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The Impact of Exercise and Energy Balance on Metabolic Control and Inflammation in Humans

Walhin, Jean-Philippe

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THE IMPACT OF EXERCISE AND ENERGY BALANCE ON METABOLIC CONTROL AND INFLAMMATION IN HUMANS

JEAN-PHILIPPE WALHIN

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department for Health

November 2013

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ABSTRACT

The aims of the work described in this thesis are to examine the impact of physical activity/exercise and energy balance on metabolic control and inflammation and specifically whether exercise has independent benefits on various health-related outcomes above a role in energy balance.

Chapter 3 examined whether a lifestyle intervention combining dietary advice with increased physical activity would further improve inflammatory markers compared to dietary advice alone and usual care in 494 patients with newly diagnosed type 2 diabetes. Motivational unsupervised diet and diet plus physical activity interventions led to reductions in inflammatory markers. Interestingly, there was no greater benefit from adding physical activity advice to dietary advice. Chapter 4 investigated whether daily vigorous-intensity exercise would counteract the metabolic changes induced by short-term overfeeding and reduced physical activity independent of any net attenuation of energy imbalance in healthy young men. The overfeeding and reduced activity model induced a state of insulin resistance, hyperinsulinaemia and altered expression of several key genes within adipose tissue. The inclusion of a daily vigorous-intensity exercise bout largely prevented these changes from taking place independent of any net effect on energy imbalance. Chapter 5 examined whether caloric restriction combined with vigorous-intensity exercise would further improve metabolic control and inflammatory markers compared to moderate-intensity exercise in middle-aged, overweight/obese men and postmenopausal women. Three weeks of caloric restriction combined with either vigorous or moderate-intensity physical exercise improved insulin sensitivity, lipid profiles and markers of inflammation. These results confirm the positive effects of combined caloric restriction and increased exercise in sedentary overweight men and women, but that exercise intensity does not seem to be so important.

In conclusion, this thesis presents reasonable evidence that exercise *per se* has a positive impact upon metabolic control and inflammation independent of energy balance during an energy surplus but that a role in contributing to the health benefits during an energy deficit are less convincing.

PUBLICATIONS

Thompson, D., Walhin, J.P., Batterham, A.M., Stokes K.A., Cooper A.R., Andrews R.C. (2013). Effect of diet or diet plus physical activity *versus* usual care on inflammatory markers in patients with newly-diagnosed type 2 diabetes: The Early ACTID randomized controlled trial. *Circulation* (under review).

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Walhin, J.P., Betts, J.A., Thompson, D. (2010) Effects of short-term overfeeding and reduced physical activity on adipose tissue gene expression. *37th Adipose Tissue Discussion Group*, Oxford, UK.

Walhin, J.P., Betts, J.A., Thompson, D. (2011) Effects of caloric restriction with vigorous *versus* moderate intensity exercise on adipose tissue gene expression. *38th Adipose Tissue Discussion Group*, Cambridge, UK.

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ABBREVIATIONS

ACTID	Activity in diabetes
AEE	Activity energy expenditure
AKT1	RAC-alpha serine/threonine-protein kinase
AKT2	RAC-beta serine/threonine-protein kinase
ALT	Alanine transaminase
AMPK	AMP-activated kinase
BCA	Bicinchoninic acid
BCAA	Branched-chain amino acid
BMI	Body mass index
BP	Blood pressure
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CHO	Carbohydrate
CI	Confidence interval
CO ₂	Carbon dioxide
CRP	C-reactive protein
Ct	Threshold cycle
CV	Coefficient of variation
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DIT	Diet induced thermogenesis
DNL	De novo lipogenesis
EDTA	Ethylenediaminetetraacetic acid
EE	Energy expenditure
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
EPOC	Excess post-exercise oxygen consumption
EtOH	Alcohol
FAS	Fatty acid synthase
FMI	Fat mass index
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT4	Glucose transporter type 4

HbA _{1c}	Glycated haemoglobin
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment for insulin resistance
HOMA- β	Homeostasis model assessment for β -cell function
HR	Heart rate
HRP	Horseradish peroxidase
HSL	Hormone-sensitive lipase
iAUC	Incremental area under the curve
IgG	Immunoglobulin G
IGT	Impaired glucose tolerance
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-6	Interleukin-6
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
ISI	Insulin sensitivity index
JAK2	Janus kinase 2
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
MCP1	Monocyte chemotactic protein
MET	Metabolic equivalent of task
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide
NEFA	Non-esterified fatty acids
O ₂	Oxygen
OGTT	Oral glucose tolerance test
PAEE	Physical activity energy expenditure
PAGE	Polyacrylamide gel electrophoresis
pAKT	Phospho protein kinase B
PAL	Physical activity level
pAMPK	Phospho 5' AMP-activated protein kinase
PAR-Q	Physical activity readiness questionnaire
PCR	Polymerase chain reaction
PDC	Pyruvate dehydrogenase complex

PDK4	Pyruvate dehydrogenase kinase isozyme 4
PI3K	Phosphoinositide 3-kinase
PPAR γ	Peroxisome proliferator-activated receptor gamma
PPIA	Peptidylpropyl isomerase A
PRO	Protein
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
RNA	Ribonucleic acid
RPE	Ratings of perceived exertion
SD	Standard deviation
SDS	Sodium dodecyl sulphate
sICAM-1	Soluble intercellular adhesion molecule-1
SREBP-1c	Sterol regulatory element binding protein 1c
STAT-3	Signal transducer and activator of transcription 3
SVF	Stromal vascular fraction
T2D	Type 2 diabetes
TAG	Triacylglycerol
TBS	Tris buffered saline
TEE	Total energy expenditure
TNF α	Tumor necrosis factor alpha
$\dot{V}CO_2$	Carbon dioxide production
VLDL	Very low-density lipoprotein
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
w/v	Weight/volume
WBC	White blood cell

CHAPTER 1

Review of Literature

1.1 Introduction

The human genome was selected in an environment where high physical activity and periods of restricted food intake were the norm. As a result, metabolic pathways that conserved energy for future food deficiency were favoured. There is strong evidence that people who are active have a lower risk of developing both cardiovascular disease (CVD) and type 2 diabetes (T2D) (Department of Health and Human Services, 1996). Today, various machines and technologies have replaced much of the physical activity that led to optimal gene expression for energy metabolism (Booth *et al.*, 2008). One of the most dramatic changes in the environment during the past 40 years has been the broad availability of relatively inexpensive, highly palatable food (Schrauwen, 2007). It is likely that most individuals intermittently experience brief periods of positive energy balance when exposed to the modern Western diet. The latest Health Survey for England (2008) suggest only 39 % of men and 29 % of women in the UK are meeting current minimum physical activity guidelines suggested by the government. Worryingly, the same survey (2011) identified 65 % of men and 58 % of women as either overweight or obese according to their Body Mass Index (BMI).

A sedentary lifestyle and a high intake of calories are conditions that can lead to a state of positive energy balance. As a result, healthy humans respond primarily by storing excess energy as triglycerides (TAG) in adipose tissue. Our ability to store excess energy as fat within the adipose tissue is impressive but at the same time can also be problematic. Excess adipose tissue increases the risk of developing a number of diseases and disorders such as T2D, CVD and the metabolic syndrome (Schrauwen, 2007). Metabolic dysfunction and chronic low-grade inflammation are likely to play a critical role in the aetiology of many chronic diseases (Duncan *et al.*, 2003). It is estimated that in England alone, 5.9 % of the population (3.1 million) is affected by T2D (Health Survey for England, 2011) while there are also thought to be around 850,000 people with

undiagnosed T2D according to the NHS. Individuals with T2D are 2-5 times more likely to develop CVD than individuals without T2D (Garcia *et al.*, 1974). Furthermore, individuals with T2D who develop CVD have a significantly poorer prognosis than individuals without T2D who develop CVD (Stevens *et al.*, 2004). According to estimates, up to 80 % of diabetic deaths are due to CVD (Biondi-Zoccai *et al.*, 2003; British Heart Foundation, 2004).

Numerous studies have achieved a reduction in body weight/fat mass through a dietary intervention which resulted in an improvement in metabolic function and markers of inflammation (Esposito *et al.*, 2004; Forsythe *et al.*, 2008; Nicklas *et al.*, 2004; Shai *et al.*, 2008). Conversely, other studies have investigated the effects of an exercise intervention on body weight/fat mass through an exercise intervention and found similar health benefits (You *et al.*, 2004; Giannopoulou *et al.*, 2005; Cho *et al.*, 2011) while others found no improvement in markers of inflammation (Marcell *et al.*, 2005). Although there is reasonable evidence that weight loss alone improves inflammatory markers and/or metabolic control, the evidence base to demonstrate whether physical activity has the potential to elicit changes in chronic inflammation and/or metabolic function over and above those gained through weight loss alone is weak.

It is generally accepted that regular physical activity and exercise are an important part of a healthy lifestyle and that physical inactivity increases the risk of disease over the long term (Booth *et al.*, 2000). It is well established that both an acute bout of exercise (Hagobian & Braun, 2006) and chronic endurance exercise training can have beneficial effects on insulin action in insulin-resistant states (Henriksen, 2002). The potential mechanisms through which physical activity leads to improvements in metabolic function and inflammatory-related benefits have yet to be elucidated. Whether it offers additional benefits compared to diet alone still remains unclear which makes interpretation of findings difficult. In order to understand and isolate how exercise exerts its effects on metabolism and whether these benefits are weight loss dependent or not; it is critically important to conduct studies that will tease

apart the effects of energy balance and weight loss from exercise/physical activity while carefully controlling energy status (Braun & Brooks, 2008).

1.2 Energy intake, energy expenditure and metabolism

Obesity plays a key role in the aetiology of chronic diseases and is fundamentally the result of an imbalance between energy intake and energy expenditure. This section aims to highlight recent changes in energy intake and expenditure and the resultant effects on macronutrient metabolism.

1.2.1 Energy intake

1.2.1.1 Caloric intake and diet composition

It is highly likely that access to food was irregular and restricted in prehistoric times while daily activities would have been dominated by outdoor physical activity consisting of hunting and foraging for food (Cooney, 2000). If the human genome evolved to support a physically active lifestyle with restricted access to food, then it is logical to presume that these individuals developed a “normal” gene expression profile (Booth *et al.*, 2008). Evolution via the survival of the fittest principle selected the genome that we still carry today. Genes predisposing to storage and energy preserving pathways would have been favoured in order to offer a better chance of survival. A modern Western diet combined with physical inactivity superimposed on these genes may have led to the current chronic disease epidemic. Even recently, there has been a noticeable increase in the proportion of fat in the British diet, while the proportion of dietary energy from carbohydrate has declined (Figure 1.1).

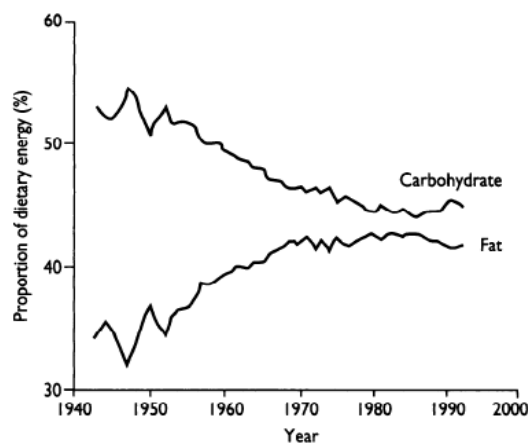


Figure 1.1: Changes in fat and carbohydrate contribution to the British diet. Taken from Prentice and Jebb (1995).

Meanwhile the average energy intake has decreased from 11.0 MJ·day⁻¹ in 1960 to 7.3 MJ·day⁻¹ in 2000 according to the National Food Survey (DEFRA). Unfortunately, the reliability of the National Food Survey findings is limited as it does not include the contribution of food consumed in restaurants for example. Paradoxically, trends in the United States appear to be different; the mean daily energy intake amongst adults has steadily increased over the course of 30 years (Table 1.1) while the relative proportion of energy from macronutrients has changed (Figure 1.2) according to the National Health and Nutrition Examination Survey (NHANES). Current research is inconclusive on potential changes in energy intake and diet composition; these discrepancies highlight the difficulty in collecting these data and possible variations between countries. However, there is no doubt that the prevalence of obesity continues to rise in affluent societies.

Table 1.1: Mean daily energy intake (in kcal) for the U.S. population, 1971 to 2000^a. Taken from Briefel & Johnson (2004).

Age/sex (years)	NHANES I 1971–74	NHANES II 1976–80	NHANES III 1988–94	NHANES 1999–2000
Both sexes				
1–2	1350	1287	1289	1511
3–5	1676	1569	1591	1622
6–11	2045	1960	1892	2025
Males				
12–15	2625	2490	2578	2460
16–19	3010	3048	3097	2932
20–39	2784	2753	2965	2828
40–59	2303	2315	2568	2590
60–74	1918	1906	2105	2123
20–74 ^b	2450	2439	2666	2618
Females				
12–15	1910	1821	1838	1990
16–19	1735	1687	1958	1996
20–39	1652	1643	1958	2028
40–59	1510	1473	1736	1828
60–74	1325	1322	1522	1596
20–74 ^b	1542	1522	1798	1877

^aOne-day intakes.

^bAge-adjusted to 2000 population.

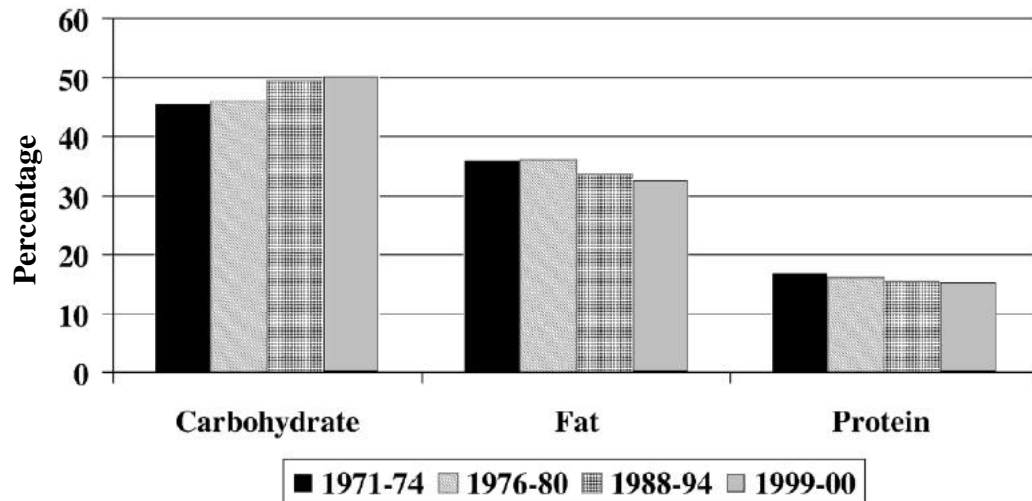


Figure 1.2: Trends in the percentage of energy from macronutrients, U.S. adults age 20-74. Data source: NHANES; age-adjusted to 2000 population. Taken from Briefel & Johnson (2004).

1.2.1.2 Carbohydrate metabolism

All carbohydrates absorbed in the small intestine must be hydrolysed into monosaccharides prior to absorption. The digestion of carbohydrates begins by the action of salivary alpha-amylase followed by the further action of pancreatic amylase in the small intestine. One of the resulting products, glucose, is stored as a multi-branched polysaccharide (glycogen) within skeletal muscle (~500 g), with alternative sources found in the liver (~100 g) and in the blood. Glycogen serves as an energy reserve that can be quickly broken down into glucose by myocytes own requirements, while hepatocytes convert glycogen into glucose to be utilised by various cell types including the central nervous system. Hepatic glycogen accounts for approximately 14 % of total stored carbohydrate while around 7 % circulates as blood glucose (Sherman, 1995). The capacity of the human body to store glucose as glycogen is limited. Glucose oxidation is assumed to be proportional to the level of glycogen stored at a given time (Schutz, 2004). Physiological pathways such as de novo lipogenesis (DNL) occur in order to deal with surplus dietary carbohydrate, once glycogen saturation has been achieved, by converting these into fatty acids where Acetyl CoA is the principal building block. This process takes place in the liver and, to a lesser extent in adipocytes (Hellerstein, 1996). Recent work is challenging this view, Hodson *et al.* (2013) has recently demonstrated that DNL is taking place

within adipose tissue in the fed state in healthy humans who are in energy balance. This pathway is believed to be thermogenically costly using approximately 28 % of the energy content of carbohydrate (Flatt, 1987; Hellerstein *et al.*, 1996). The excretion of glucose in the urine (glycosuria) does not usually take place as the glomerular barrier of the kidneys is able to reabsorb all of the filtered glucose back into the bloodstream. It is usually a consequence of elevated blood glucose levels often seen in untreated diabetes (Ballantyne *et al.*, 1977).

1.2.1.3 Fat metabolism

Triglyceride digestion occurs almost exclusively in the small intestine. The major digestive enzyme in this process is pancreatic lipase which breakdowns triglycerides (TAG) into fatty acids and monoglyceride. Following the emulsification process, micelles are formed containing the products of fat digestion. The content of micelles become available to diffuse across the intestinal lining where fatty acids and monoglycerides are resynthesized into triglycerides before being released into the systemic circulation as chylomicrons via the lymphatic system. The fatty acids of plasma chylomicrons are released, mainly within adipose tissue capillaries, by the action of endothelial lipoprotein lipase (LPL). The released fatty acids then enter adipocytes, hepatocytes or myocytes and combined with α -glycerol phosphate (synthesised in the adipocytes from glucose metabolites) to be stored as TAG. The liver acts as a major organ for fatty acids, processing chylomicrons into various lipoproteins, in particular very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Glucose entering the liver can either be stored as glycogen or transformed into α -glycerol phosphate and fatty acids, which are then used to synthesise TAG, as in adipose tissue. Some of the fat synthesised from glucose in the liver is stored there, but most of it is released as VLDL. The liberation of fatty acids or fat mobilisation takes place in adipose tissue when other tissues require it, for instance during exercise or following an overnight fast. The process of fat mobilisation is catalysed by the enzyme hormone-sensitive lipase (HSL) and the resulting non-esterified fatty acids (NEFA) enter the plasma only from adipose tissue. NEFA are not water-soluble and are carried in plasma bound to albumin. NEFA are taken up by, among other tissues, skeletal muscle

and the liver. In each of these tissues, they may enter the pathway of β -oxidation, or they may be used for the synthesis of TAG. An excessive intake of fat can be stored as TAG; this is by far the biggest and most efficient store of energy within the human body.

1.2.1.4 Protein metabolism

Proteins are broken down to peptide fragments by pepsin in the stomach and by trypsin and chymotrypsin, the major proteases secreted by the pancreas, in the small intestine. These fragments are further digested to free amino acids by carboxypeptidase and aminopeptidase. The free amino acids then enter the epithelial cells before being released into the interstitial fluid and then into the systemic circulation. Most amino acids are sufficiently water-soluble to circulate freely in plasma except for tryptophan which is bound loosely to albumin for transport. Amino acids cross the cell membrane via specific transporters. As well as being one of the building blocks of body tissue, protein can also serve as a fuel source. Amino acids can provide gluconeogenic precursors for hepatic glucose production (Butterfield, 1990). Only the branched chain amino acids (BCAA) along with glutamate, asparagine and aspartate are available for oxidation within skeletal muscle (Rennie & Tipton, 2000). Some studies suggest that adipose tissue is also capable of oxidising significant amounts of BCAAs (Rosenthal *et al.*, 1974; Tischler & Goldberg, 1980). In fact, very little amino acids are lost from the body intact and most of the amino acids we ingest are ultimately oxidised. Amino acid oxidation contributes around 10-20 % of the total oxidative metabolism of the body under normal conditions. All other amino acids must be transaminated into either glutamate or alanine before subsequent deamination in the liver, at which point the resultant carbon can be oxidised and the amino group disposed of as urea (Felig and Wahren, 1971). Although protein represents a source of energy that can be drawn upon during starvation, there is no specific storage form of protein. All proteins have some function other than storage of energy. In evolutionary terms, we can expect that other sources of energy such as glycogen and TAG will be favoured. Amino acids can be oxidised just as glucose and fatty acids can; however, unlike fatty acids, amino acids can be converted into glucose. This gives the body's protein store (muscle mass) a special role during starvation, when the body must

maintain the availability of circulating glucose despite the absence of external carbohydrate supply. On the other hand, an excess of amino acids might stimulate muscle protein synthesis to a small extent over a short period of time (Bennet *et al.*, 1989). However, this increased muscle protein synthesis will then be inhibited (Bohe *et al.*, 2001). Overfeeding protein does not increase the size of the lean body mass long term and amino acids supplied in excess are simply oxidised (Motil *et al.*, 1981; Price & Mitch, 1994) and their carbon skeletons used for fuel or stored as fat.

1.2.1.5 Alcohol metabolism

The average human digestive system produces approximately 3 g of ethanol per day through fermentation of its content (Zakhari, 2006). As a result, catabolic degradation of ethanol is essential to humans. Alcohol is primarily absorbed from the small intestine; however, alcohol cannot be stored and therefore, the body must metabolise it to remove it. Alcohol can only be oxidised in the liver where enzymes are found to initiate the process. Ethanol is oxidised into acetaldehyde which is itself further oxidised to acetate and finally CO₂ and water through the citric acid cycle. A number of metabolic effects from alcohol are directly linked to the production of an excess of NADH and acetaldehyde (Zakhari, 2006). Excess NADH may be used as a reducing agent in two pathways; one to synthesise glycerol and the other to synthesise fatty acids. Acetate is also metabolised to Acetyl CoA, which is involved in lipid and cholesterol biosynthesis (Lieber, 1976).

1.2.2 Energy expenditure

1.2.2.1 Changes in physical activity

Humans evolved in an environment where survival necessitated a large amount of physical activity and as a result, the cardiovascular system and musculoskeletal systems evolved to meet the requirements of these surroundings. The unique ability to expend large amounts of energy in order to sustain prolonged periods of physical activity is an achievement that no other primate and few other mammals are able to perform (Archer & Blair, 2011). However, this is only feasible because our physiology has evolved to favour

metabolic pathways that conserve energy for future requirements. The shift from hunting and gathering to agriculture during the Neolithic era facilitated the transition to a less energetically way of life (Weisdorf, 2005). Nevertheless, this was still enough to maintain health and prevent chronic diseases. For most of human history, infectious diseases were the leading cause of death rather than chronic diseases. Yet, with time, the demands placed on the body diminished resulting in the current chronic disease epidemic we are now facing.

Hippocrates observed that “sudden death is more common in those who are naturally fat than in the lean” in 400 BC, this observation remained unexplained for many centuries. Although the work of Dr WA Guy on the health benefits from physical activity was conducted over a century ago, the main body of evidence came from the work conducted by Professor Jerry Morris. In a landmark study, he showed that sedentary bus drivers had a higher death rate due to CVD compared to conductors, whose job specifically required them to be more physically active (Morris *et al.*, 1953). Systematic research on the relationship between physical activity and health only began in the second half of the 20th century, and until the mid-1980s studies relied on self-reported physical activity questionnaires. Because of this, there are few baseline data on physical activity patterns against which to compare secular trends. Durnin (1992) presented some indirect evidence that energy expenditure amongst UK adolescents must have declined over time by highlighting the decline in energy intake from the 1930s to the 1980s, with no parallel change in body mass. Television viewing is one exception for which we have historical data available. According to the General household survey (1994), the average person in England watched over 26 hours of television a week in 1994, compared with 13 hours in the 1960s.

The latest Health Survey for England (2008) suggest only 39 % of men and 29 % of women in the UK are meeting current minimum physical activity guidelines suggested by the government which involves 30 minutes or more of moderate or vigorous activity (20 minutes, 3 times a week) on at least 5 days a week. Conversely, Westerterp & Speakman (2008) suggests that physical activity energy expenditure has not decreased since the 1980s while the obesity rates

have continued to soar suggesting that an increase in energy intake must be to blame. Whether changes in physical activity patterns, dietary habits or a combination are to blame for the current obesity epidemic and the associated rise in chronic diseases remains disputed.

1.2.2.2 Exercise metabolism

During exercise, large quantities of fuel are metabolised in order to meet energy requirements to support muscle contraction. Various substrates can be utilised such as plasma glucose, skeletal muscle and hepatic glycogen, fatty acids as well as amino acids.

Skeletal muscles convert chemical energy into mechanical work and therefore use the majority of energy during exercise. During submaximal steady-state exercise, the proportion of carbohydrates and fat being oxidised depends on a number of factors such as substrate availability, training status, exercise intensity and gender (Arkinstall *et al.*, 2004). During high-intensity exercise, glycogen is the main source of fuel (Romijn *et al.*, 1993). A low-glycogen state leads to an increased fat metabolism and amino acids are released in the circulation from muscle protein breakdown (Blomstrand & Saltin, 1999). Interestingly, muscle glycogen levels do not majorly decrease during short-term fasting (Vendelbo *et al.*, 2012).

The liver plays a key role by supplying additional plasma glucose during exercise through the breakdown of its own glycogen into glucose 6-phosphate in order to supply glucose to other tissues. Fasting dramatically affects hepatic glycogen levels and after 24 h, liver glycogen has decreased by ~65 % (Magnusson, 1992). The increase in glucose utilisation by the muscle during exercise challenges glucose homeostasis and the liver is the main source of systemic glucose when fasted (Wahren *et al.*, 1971). Glucose requirements are met during the early stages of exercise through glycogenolysis (Ahlborg & Felig, 1982). Gluconeogenesis mainly takes place in the liver by producing glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol and certain amino acids (Coggan, 1991). Prolonged exercise, restricted carbohydrate intake as well as increased exercise intensity will increase the rate

of gluconeogenesis. Towards the end of prolonged exercise, the production of glucose through gluconeogenesis can account for up to 20-50 % of total glucose release (Ahlborg & Felig, 1982).

Triglyceride stores are a major source of fuel for low- and moderate-intensity exercise and daily physical activity. The large increase in adipose tissue lipolysis provides glycerol available to the liver as well as non-esterified fatty acids (NEFA) which is used as an additional source of energy to the exercising muscle. The availability of NEFA to the muscles also reduces glucose oxidation. NEFA oxidised during exercise comes from 2 sources: triglyceride stored within the adipose tissue and within skeletal muscle. NEFA concentrations increase 2-3 fold during exercise compare to resting values, but the overall contribution of NEFA towards energy expenditure is limited to ~60 % (Frayn, 2010).

The liver plays a key role in amino acid oxidation as it is the only organ capable of eliminating the nitrogen from amino acids by synthesising urea. Amino acid catabolism predominantly takes place in the liver with the exception of BCAAs (leucine, isoleucine and valine) as well as glutamate, aspartate and asparagine which are largely catabolised by the skeletal muscles. Sustained exercise stimulates amino acid oxidation, especially of the BCAAs. As a result, ammonia production is proportional to exercise intensity (Rennie & Tipton, 2000). Although the contribution of amino acids/protein towards the energy needs during exercise is not as important as either carbohydrate or fat, it contributes significantly towards total exercise calories (Lemon & Nagle, 1981).

1.3 Metabolic health and inflammation

1.3.1 Link between adipose tissue and inflammation

The global obesity epidemic currently taking place in many Westernised parts of the world is fast becoming a serious public health problem due to the resulting increased risks of developing chronic diseases such as type 2 diabetes, metabolic syndrome, cardiovascular diseases and certain cancers according to the World Health Organization. Exercise and physical activity make a major

contribution to total energy expenditure and therefore energy balance. Western lifestyles are characterised by chronically low levels of physical activity and excessive caloric intake, resulting in positive energy balance (Hill & Wyatt, 2005; Uauy & Diaz, 2005), and an accumulation of triacylglycerol (TAG) within adipocytes. Adipose tissue has evolved to store energy efficiently for periods of reduced caloric intake. The adipose tissue capacity to store excess energy as TAG is tremendous, it can increase manifold to >80 % of body weight in obese people (Thompson *et al.*, 2012) but the adipose organ is badly equipped to cope with this increased load. Although adipocytes typically constitutes 80-90% of adipose tissue volume (Thompson *et al.*, 2012), they only constitute 60-70% of the total cell population. The remainder constitutes the stroma-vascular fraction (SVF) which includes endothelial cells, fibroblasts, pre-adipocytes and immune cells such as macrophages, more so in obese people (Langin *et al.*, 2009).

Long-term whole body energy balance is reflected in the storage of TAG within the adipose tissue as the two are tightly linked. The body's ability to store glycogen within the liver (~100 g) and skeletal muscle (~500 g) is limited, as a result, long term imbalances between energy intake and energy expenditure will be reflected in the variation in TAG stored within adipocytes. Adipose tissue plays a key role in lipid metabolism; it is a key site of absorption of TAG from the circulation and is the only organ capable of realising large amounts of NEFA in the circulation as a source of energy for other tissues such as skeletal muscle and liver. The coordination between fat storage and mobilisation are tightly controlled (Frayn *et al.*, 1995; Evans *et al.*, 2002) and post-prandial state involves the whole tissue, not just adipocytes, as shown by the dynamic regulation of adipose tissue blood flow (Summers *et al.*, 1996; Summers *et al.*, 2001). Adipose tissue is a highly vascularised tissue; in a preprandial state, adipose tissue blood flow is greater than the blood flow in resting skeletal muscle (Bickerton *et al.*, 2007). The regulation of these processes must be regulated in reflection of whole body energy balance. Catecholamines regulate fat mobilisation and this process seems to be blunted in obese people (Horowitz & Klein, 2000) and weight loss does not appear to improve this impaired lipolysis (Blaak *et al.*, 1994). This blunted response in fat mobilisation in

reaction to catecholamines is also a characteristic of childhood obesity (Bougnères *et al.*, 1997; Enoksson *et al.*, 2000) which also occurs in adipocytes among first-degree relatives of obese subjects (Hellstrom *et al.*, 1996). Jocken *et al.* (2008) showed that the beta-adrenergically mediated lipolytic response of obese men was blunted systematically and in abdominal subcutaneous adipose tissue compared to lean men. This could have an impact on the development or maintenance of TAG stores and obesity.

Other factors such as glucocorticoids have also been shown to up-regulate lipolysis and thus the release of fatty acids (Samra *et al.*, 1998). Interestingly, insulin has been shown to increase lipogenesis in both differentiated and undifferentiated adipocytes (Gathercole *et al.*, 2011). It is likely that insulin is mainly responsible for diurnal variations in lipolysis which is elevated following an overnight fast and blunted in a postprandial state. The increase in lipogenesis following feeding is achieved via the increase activity of lipoprotein lipase (LPL) in adipose tissue capillaries following the increasing levels of insulin. This results in the hydrolysis of circulating TAG (Wang *et al.*, 2009) which produces an excess of fatty acids, some of which will be taken up and stored within the adipocytes (Saleh *et al.*, 1998). The amount of fatty acids taken up by the adipose tissue for storage varies (Evans *et al.*, 2002); this leads to a proportion of fatty acids called the “spillover” which has not been taken up by the adipose tissue. Research suggests that these metabolic responses are more tightly regulated in lean healthy individuals compared to obese, insulin resistant individuals or people who suffer from type 2 diabetes (Coppack *et al.*, 1992; Tan *et al.*, 2005; Tan *et al.*, 2006). This has been described by Frayn, Tan & Karpe (2007) as impairment in “metabolic flexibility” or in other words a reduction in the normal response of the adipose tissue in response to nutrient intake leading to a “fat overflow”. As a result, other tissues might be exposed to an excess of fatty acids, especially following feeding (Frayn *et al.*, 2002).

While this lack of “metabolic flexibility” might partly explain a decrease in insulin sensitivity in skeletal muscle or liver, a glucose transporter type 4 (GLUT 4) knockout mouse model suggest there might be other mechanisms involved. Abel *et al.* (2001) created a GLUT 4 knockout mouse model where an

adipose-selective reduction in GLUT 4 was achieved. Interestingly, this resulted in a state of insulin resistance in skeletal muscle and liver despite GLUT expression being preserved in muscle. The knock-out of GLUT 4 within adipocytes would have resulted in an impairment of fatty acids absorption by the adipose tissue as glycerol 3-phosphate resulting from glucose metabolism is required for fatty acid esterification. Surprisingly, the insulin resistance reported was not associated with an accumulation of TAG within the liver and skeletal muscle.

A positive energy balance resulting from an excessive caloric intake and lack of physical activity results in hypertrophy of the adipose tissue, which is common in patients with type 2 diabetes in an attempt to store the energy surplus. A possible explanation is that larger, “triglyceride-filled” adipocytes are less metabolically “flexible” or have an impaired response to nutrient intake. In humans, large adipocytes are linked with an increased risk of developing type 2 diabetes (Paolisso *et al.*, 1995; Weyer *et al.*, 2000). The lack of adipose tissue “flexibility” is also apparent in obese people where adipose tissue blood flow appears to be disturbed and this appears to be closely linked with insulin resistance (Karpe *et al.*, 2002; Virtanen *et al.*, 2002).

Frayn, Tan & Karpe (2007) propose that a stimulus such as expansion of the adipocyte beyond its normal range might result in a metabolic “stress” which would induce the release of chemical signals that would attract monocytes (i.e., the initiation of an inflammatory process). Adipose tissue is not just a site of energy storage and acts as an endocrine organ capable of secreting growth factors and adipokines (Rajala & Scherer, 2003), which might act as a signal to alter the metabolism of other tissues. The authors conclude by suggesting that an increased fat mass might result in a metabolic inflexibility of the adipose tissue, compounded by inflammatory changes and the release of various adipokines resulting in the altered metabolism observed in other tissues.

1.3.2 Link between inflammation and chronic diseases

Adipose tissue is an endocrine organ capable of secreting a number of proteins, collectively called adipokines. These include various hormones and cytokines,

such as adiponectin and leptin, all of which can have an impact on energy homeostasis, metabolism, immunity and inflammation. As a result, the adipose tissue is capable of responding to signals from other organs by regulating the secretion of these adipokines. An increase in adipocyte size results in an altered secretory profile of some of these proteins, which can negatively impact other systems (Bluher *et al.*, 2002). Almost all known adipokines are dysregulated in conditions such as type 2 diabetes, obesity, metabolic syndrome and atherosclerosis. Many adipokines involved in the aetiology of insulin resistance and inflammation (e.g., TNF α , IL-6) are up-regulated with increased adiposity while other insulin-sensitising or anti-inflammatory adipokines (e.g., Adiponectin) are down-regulated as fat mass increases resulting in metabolic complications (Maury & Brichard, 2010; Figure 1.3).

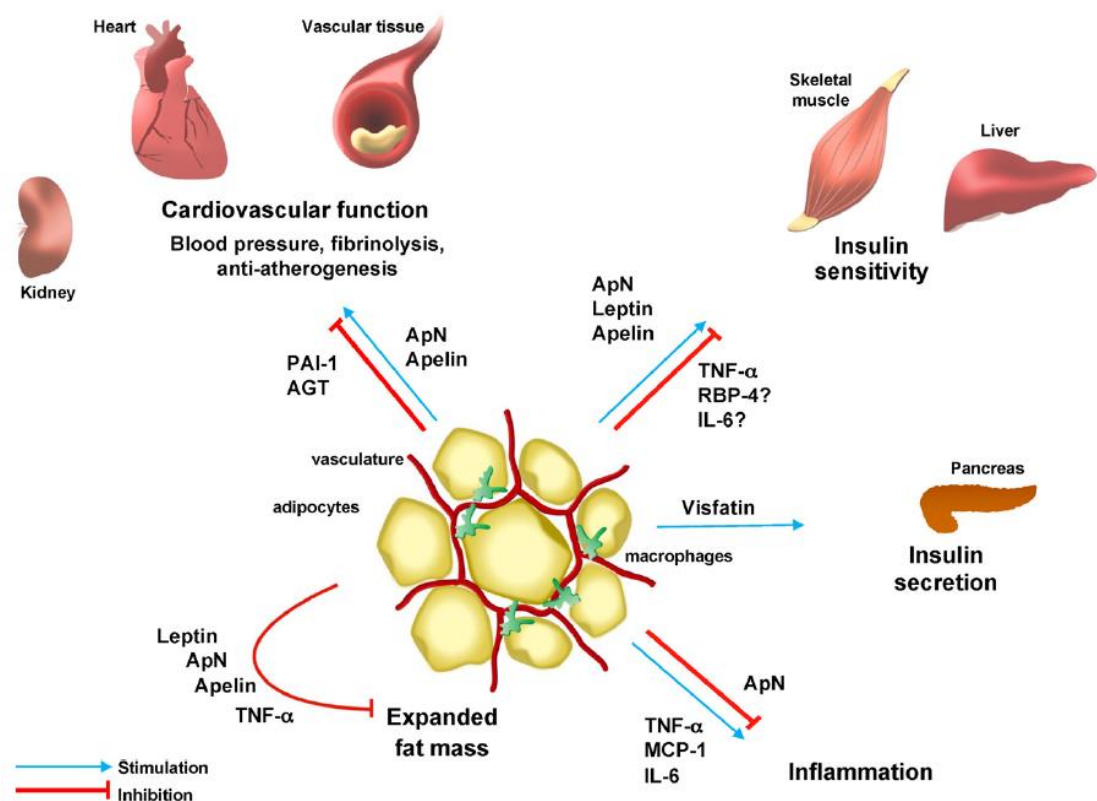


Figure 1.3: Adipokines involved in the pathogenesis of the metabolic syndrome. ApN= Adiponectin; PAI-1= plasminogen activator inhibitor type 1; AGT= angiotensinogen; TNF- α = tumor necrosis factor- α ; RBP-4= retinol-binding protein-4; MCP-1= monocyte chemoattractant protein-1; IL-6= interleukin-6. Taken from Maury & Brichard (2010).

Adipose tissue is unusual as a secretory organ as different fat depots (subcutaneous *versus* visceral) have different metabolic profiles and also differ in their adipokines secretion patterns. An increase in abdominal fat is usually linked with negative effects on metabolic function and mortality (Pischon *et al.*, 2008) while an increase in lower-body fat is linked with protective effects (Manolopoulos *et al.*, 2010). Differences in secretion may have a local impact on the adipose tissue and intra-peritoneal fat may directly impact the liver. It is worth remembering that other organs might contribute to the systemic concentrations of certain adipokines.

Enlarged adipocytes secrete some adipokines such as monocyte chemoattractant protein-1 (MCP-1) in excess, resulting in an infiltration of monocytes/macrophages. Obesity has been linked with an increase in macrophage infiltration (Weisberg *et al.*, 2003; Xu *et al.*, 2003); both BMI and adipocyte size have been shown to be correlated with macrophage infiltration in humans (Weisberg *et al.*, 2003). Canello *et al.* (2005) showed that following bypass surgery, morbidly obese patients experienced a drastic weight reduction (Δ 22.1 kg), a significant decrease in macrophage number (\sim -12 %) with the remaining macrophages staining positive for the anti-inflammatory protein interleukin-10 (IL-10). Adipose tissue of obese patients shows that the majority of macrophages gather in “crown-like structures” which surround dead adipocytes (Figure 1.4; Canello *et al.*, 2005; Cinti *et al.*, 2005). The accumulation of macrophages within adipose tissue is partly responsible for some of the complications linked with human obesity. It is linked with fibro-inflammatory lesions in the liver, arterial dysfunction and reduced insulin sensitivity in obese subjects (Canello *et al.*, 2006; Apovian *et al.*, 2008)

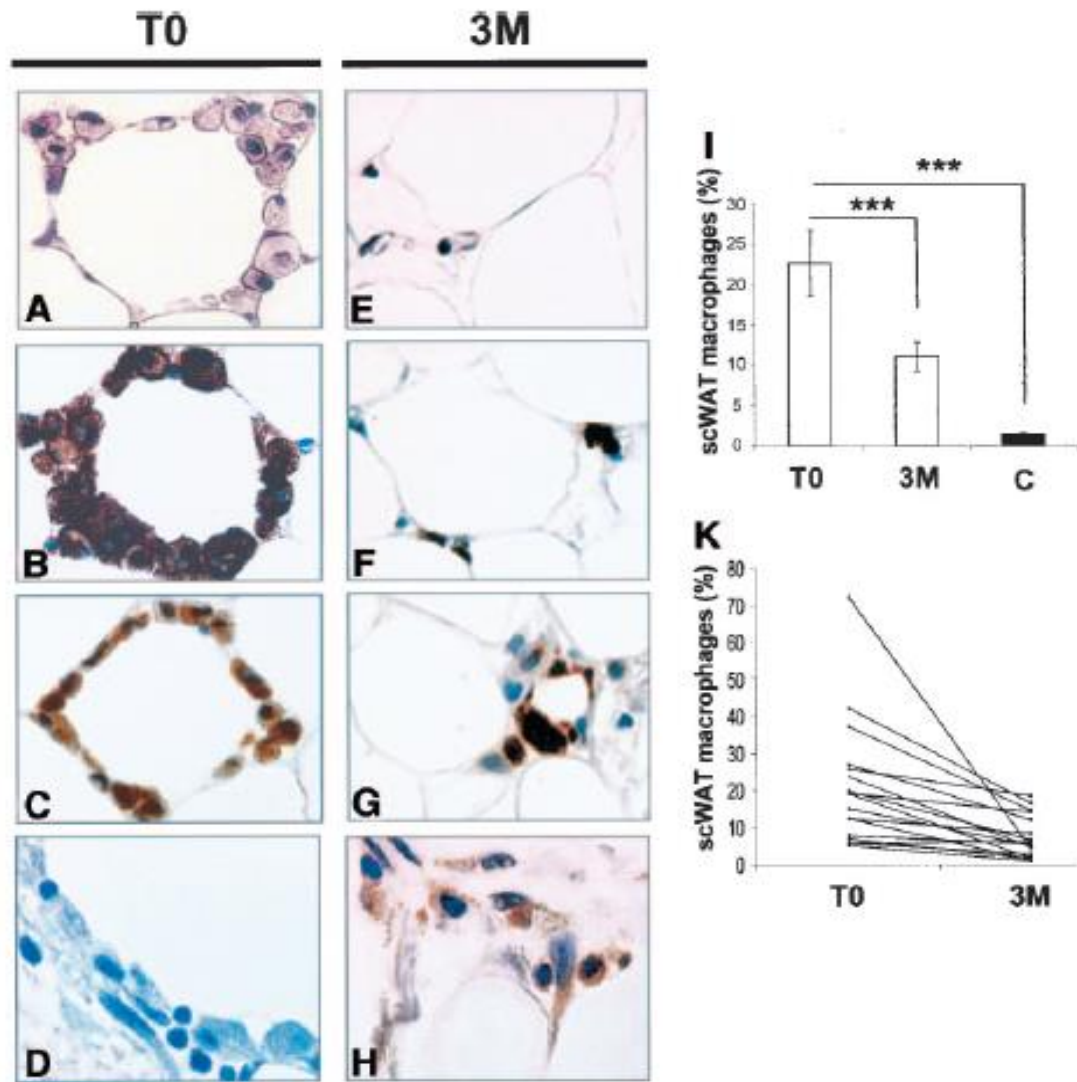


Figure 1.4: Macrophage infiltration of subcutaneous adipose tissue in obese subjects before (T0) and 3 months after (3M) weight loss surgery. A–D: The morphology of subcutaneous adipose tissue of one representative morbidly obese study subject (BMI $50 \text{ kg} \cdot \text{m}^{-2}$) is shown. Infiltrating macrophages formed a typical crown. Immunopositivity for HAM56 (B; X100) and CD68 (C; X100). D: Lack of immunostaining for IL-10 (X100). Adipocytes tested negative to all these markers. E–H: Immunomorphology of the subcutaneous adipose tissue sample obtained 3 months after surgery. Routine Mayer’s hematoxylin and eosin staining (E; X100): absence/disorganization of macrophage crowns. Immunopositivity for HAM56 (F; X100) and CD68 (G; X100). H: Immunostaining in resting macrophages for IL-10 (X100). Percentages of macrophages were estimated in subcutaneous adipose tissue specimens before/after weight loss and in lean subjects, $***P < 0.001$ (I). Individual decreases in percentage of macrophages in 17 morbidly obese subjects 3 months after weight loss surgery ($***P < 0.001$) (K). Taken from Cencello *et al.* (2005).

1.3.3 Inflammatory markers/Adipokines

1.3.3.1 CRP

Of the inflammatory measures that are readily available, serum concentration of C-reactive protein (CRP) is commonly used and appears to consistently predict the risk of cardiovascular disease. CRP is a hepatic protein which increases in response to trauma, inflammation or infection. It is secreted in response to systemic concentrations of IL-6, which is mainly produced by the adipose tissue (Mohamed-Ali *et al.*, 1997), and is a useful indicator of the inflammatory state of the body as it has a relative long half-life compared to other inflammatory markers (Vigushin *et al.*, 1993). Research suggests that CRP is the most accurate inflammatory marker to predict future risk of cardiovascular events (Schillinger *et al.*, 2003; Rutter *et al.*, 2004; Schulze *et al.*, 2004), risk of death (Stehouwer *et al.*, 2002), atherosclerotic progression (Kang *et al.*, 2004) and development of peripheral vascular disease (Yu *et al.*, 2004). A meta-analysis by Kaptoge *et al.* (2010) investigated whether CRP concentrations were associated with risk of vascular and non-vascular outcomes (cancers and respiratory diseases) under different conditions. The authors concluded that CRP concentrations were associated with future risks of cardiovascular disease, ischemic stroke and deaths from vascular and non-vascular disease. Higher BMI has been shown to be linked with higher concentrations of CRP in young adults (17 to 39 years old), thus confirming that low-grade chronic inflammation is a feature of obesity (Visser *et al.*, 1999). Bariatric surgery in morbidly obese patients has been shown to significantly reduce CRP concentrations (Vazquez *et al.*, 2005) but less so in insulin-resistant patients (Holdstock *et al.*, 2005), indicating that CRP might be primarily dependent upon insulin sensitivity rather than energy supply. Circulating CRP concentrations increase continuously across the spectrum of fasting glucose concentrations, beginning at the lowest quartile of normal fasting glucose (Aronson *et al.*, 2004).

1.3.3.2 IL-6

Interleukin-6 (IL-6) is a pleiotropic cytokine with both anti- and pro-inflammatory properties which is primarily secreted by white blood cells but also by endothelial cells and myocytes (Fischer *et al.*, 2004). The adipose tissue is also a particularly important source of IL-6 secreting up to 30 % of circulating levels of IL-6 in healthy subjects (Mohamed-Ali *et al.*, 1997). Visceral fat has been shown to produce substantial amounts of IL-6 in obese individuals (Fontana *et al.*, 2007). Like CRP, IL-6 is secreted in response to trauma, inflammation or infection. As stated earlier, IL-6 stimulates the hepatic secretion of CRP and other acute phase proteins (Frayn & Stanner, 2005). There is growing evidence that IL-6 is also a valid predictor of future cardiovascular events as well as potentially playing a direct role in the progression of atherosclerosis (Ridker *et al.*, 2000; Madan *et al.*, 2008). It has been suggested that IL-6 might be linked with insulin resistance (Lee *et al.*, 2009); the adipose tissue of insulin-resistant moderately obese women has been shown to express significantly more IL-6 than their insulin-sensitive counterparts (McLaughlin *et al.*, 2008).

1.3.3.3 Adiponectin

Adiponectin is dramatically reduced in obesity (Baranova *et al.*, 2007) as increased adiposity is associated with lower serum concentrations of adiponectin (Rajala & Scherer, 2003). This adipokine has direct anti-inflammatory effects (Rajala & Scherer, 2003; Pischon *et al.*, 2004; Shimada, Miyazaki & Daida, 2004; Schulze *et al.*, 2004). Adiponectin is synthesized in substantial amounts exclusively by adipocytes (Baranova, 2008). In patients with type 2 diabetes compared with subjects without diabetes, low adiponectin concentrations correlate strongly with insulin resistance (Weyer *et al.*, 2001). Adiponectin has been shown to increase both hepatic and peripheral insulin sensitivity (Fasshauer & Paschke, 2003). In insulin-resistant rodents, the administration of recombinant adiponectin has led to increased insulin sensitivity (Yamauchi *et al.*, 2001).

1.3.3.4 sICAM-1

Soluble adhesion molecules have a non-adipose origin, being derived primarily from endothelial cells and leukocytes. These molecules promote the attachment of leukocytes to the vessel wall in the early stages of atherosclerosis and subsequent migration. Soluble adhesion molecules, mainly shed from the surface of the endothelium are an indicator of cellular expression (Price and Loscalzo, 1999). Such markers provide information on inflammation within the vasculature (Pradhan *et al.*, 2002; Blankenberg *et al.*, 2003). It is noteworthy that soluble Intercellular Adhesion Molecule-1 (sICAM-1) independently predicts the risk of cardiovascular disease (Shai *et al.*, 2006) and the risk of stroke in people with Type 2 Diabetes (Kanai *et al.*, 2008). Following 3 weeks of caloric restriction and increased physical activity, sICAM-1 was significantly reduced in obese men (Roberts *et al.*, 2006). The analysis of ICAM-1 in the plaques obtained from coronary artery bypass suggests that it is increased with smoking status and altered in diabetes.

1.3.3.5 Leptin

Leptin is an adipokine that is predominantly expressed in the adipose tissue and is involved in the regulation of feeding behaviour. Leptin levels are correlated with adiposity, decreased following caloric restriction and increased with re-feeding (Das, 2001). The primary effect is that adipocyte-derived leptin acts on the hypothalamus to centrally suppress feeding and fatty acid metabolism (Baranova, 2008). Interestingly, obese patients tend to have higher circulating levels of leptin explained by a relative resistance of their neurons to the effect of leptin (Sahu, 2003). Leptin and insulin compete for the same signaling molecules, in particular, JAK2/STAT-3 and phosphoinositide 3-kinase (PI3K; Baranova, 2008). As a result, an increase in leptin reduces insulin efficiency (Benomar *et al.*, 2005). The relationship between leptin and insulin sensitivity is complex as it appears to have an impact on insulin action in peripheral tissues, pancreatic β -cells and blood vessels (Ronti *et al.*, 2006; Seufert, 2004). Overfeeding studies consistently showed an increase in serum leptin concentrations (Joosen *et al.*, 2006; Brons *et al.*, 2009).

1.4 Metabolic control, inflammation and physical activity

1.4.1 Epidemiological studies

Many epidemiological studies have investigated the effects of physical activity on metabolic outcomes and markers of inflammation. A selection of these studies is discussed in this section. In 2003, Rennie *et al.* published data from the Whitehall II study of civil servants that included 5153 white European participants (age 45-67 years). Cross-sectional relationships between the metabolic syndrome and moderate and vigorous physical activity were examined. Cardiovascular fitness and BMI were examined as possible mediators of the observed association. Measures of HDL cholesterol, systolic blood pressure, 2-hour glucose values following an oral glucose tolerance test (OGTT), fasting TAG and waist-hip ratio were collected. Self-reported leisure-time physical activity was categorised into separate moderate and vigorous activity categories. Resting heart rate and BMI were used to estimate cardiovascular fitness and body fatness, respectively. Participants in the most adverse sex-specific quintile for 3 or more of these risk factors were classified as having the metabolic syndrome. The odds ratios (95 % CI) for having the metabolic syndrome in the top categories of vigorous and moderate activity were 0.52 (95 % CI: 0.40, 0.67) and 0.78 (95 % CI: 0.63, 0.96) respectively, adjusted for age, sex, smoking, alcohol intake, socioeconomic status, and other activity. Adjustment for BMI and resting HR substantially attenuated both of the above associations. The authors concluded that vigorous and moderate physical leisure-time activity are each associated with decreased risk of being classified with the metabolic syndrome independently of age, smoking, and high alcohol intake. Both activities (moderate and vigorous) may be beneficial to the metabolic syndrome cluster of risk factors among middle-aged populations. Increased cardiovascular fitness and reduced BMI may be key mediators of this association for both intensities of activity.

Demakakos *et al.* (2010) examined whether there was an association between small amounts of low-intensity physical activity and reduced risk of developing T2D in 7466 individuals aged 50 or over (56 % women). Participants were followed for a mean of 45 months. Physical activity was assessed at baseline via

interview. Vigorous/moderate-intensity physical activity at least once a week was associated with reduced risk of developing type 2 diabetes but low-intensity physical activity at least once a week was not after adjustment for all covariates. However, age-stratified analysis showed that low-intensity physical activity at least once a week was associated with reduced risk of developing type 2 diabetes for those aged 70 years and over. The authors concluded that any type of physical activity reduced the risk of developing T2D compared to physical inactivity in adults aged 70 years and over. Interestingly, in adults aged 50 to 69 years, physical activity needed to be moderate/vigorous (as opposed to low) to reduce the risk of developing T2D.

Fischer *et al.* (2007) interviewed 84 healthy non-diabetic men and women on the amount of leisure-time physical activity they took part in. Adiponectin, CRP and IL-6 were measured. Participants were separated into 4 categories; active and non-obese, inactive and non-obese, obese and inactive or obese and active. Participants with a waist-hip ratio greater than 0.90 for males or 0.85 for females or a BMI > 30 kg m⁻² were classified as obese. CRP and IL-6 were associated with leisure time physical activity independent of obesity and *vice versa*. Adiponectin was associated with obesity independent of leisure time physical activity but not with leisure time physical activity independent of obesity. The authors concluded that the benefits of leisure time physical activity on inflammation are independent of its impact on obesity and that they might in fact be additive.

On the whole, epidemiological studies seem to suggest that physical activity is beneficial in reducing markers of inflammation and reducing the risk of developing chronic diseases such as the metabolic syndrome and T2D. Importantly, these are associations studies and not causal

1.4.2 Physical activity intervention studies and metabolic control/inflammation

Many intervention studies have attempted to investigate the effects of exercise/physical activity on several metabolic/inflammatory markers. Some studies have looked at the effect of exercise *per se*, others have looked at exercise combined with caloric restriction/weight loss. Improvements in metabolic and inflammatory markers seem to be increased with greater weight/fat loss whether this is induced by increased physical activity, caloric restriction or a combination of both. Although there is reasonable evidence that weight loss alone improves metabolic and inflammatory markers, it remains unclear whether physical activity has the ability to improve metabolic function and chronic inflammation independent of changes in weight/fat mass. Current findings seem to suggest that lifestyle interventions combining caloric restriction and increased physical activity are most beneficial. Studies looking at the effect of exercise alone or exercise combined with caloric restriction, including participants who overweight/obese, are summarised in Table 1.2. Exercise studies including participants who are insulin resistant or suffer from type 2 diabetes are summarised in Table 1.3. Studies combining exercise and caloric restriction, including participants who are insulin resistant or suffer from type 2 diabetes are summarised in Table 1.4. Only recent studies are presented in this section; the list is non-exhaustive.

Table 1.2: Interventions investigating the effects of exercise alone or exercise and caloric restriction on metabolic control and inflammation in overweight/obese participants. CR= Caloric restriction, VLCD= Very-low calorie diet, HR=heart rate, AUC= Area under the curve

Author	Duration	Study population	Intervention	Body mass change	Outcome
Arsenault <i>et al.</i> (2009)	6 months	267 overweight/obese women	Exercise 3-4 times/week, 50 % $\dot{V}O_{2max}$	-1.4 kg	Lipid profiles and inflammatory markers did not improve
Brochu <i>et al.</i> (2009)	6 months	107 overweight/obese postmenopausal women	2 groups: 1/CR 2/CR + resistance exercise	1/-5.1 kg 2/-5.8 kg	↓ in TAG, fasting insulin, CRP, no difference between groups
Campbell <i>et al.</i> (2009)	12 months	202 sedentary, middle-aged, overweight/obese men and women	2 groups: 1/moderate to vigorous aerobic exercise 2/control	1/-1.6 kg 2/+0.3 kg	No reduction in CRP
Imayama <i>et al.</i> (2012)	1 year	439 overweight/obese postmenopausal women	4 groups: 1/CR 2/aerobic exercise 3/diet + exercise 4/control	1/-8.5% 2/-2.4 % 3/-10.8 % Compared to control	CR weight loss diet with or without exercise reduces markers of inflammation (CRP & IL-6)
Izadpanah <i>et al.</i> (2011)	2 weeks	22 overweight/obese adults	High-fibre, low-fat diet + daily 45-60 min of exercise, 70-85 % max HR	Δ in average BMI from 33.5 to 32.2 $kg\cdot m^{-2}$	↓ in TAG, cholesterol, HDL cholesterol, glucose, insulin, HOMA-IR & leptin
Kohut <i>et al.</i> (2006)	10 months	97 older adults	2 groups: 1/flexibility and resistance exercise 2/ aerobic exercise	No change in body weight	↓ in CRP & IL-6 in aerobic exercise group. TNF- α ↓ in both groups.
Olson <i>et al.</i> (2007)	1 year	28 overweight women	Resistance exercise at least twice a week. 2 groups: 1/resistance 2/control	1/+2.1 kg 2/-0.7 kg	No changes in BP, lipids, glucose or insulin. CRP↓, Adiponectin↑ in resistance group. IL-6 did not change.
Thompson <i>et al.</i> (2010)	6 months	41 sedentary middle-aged men	2 groups: 1/30-60 min exercise, 50-70 % $\dot{V}O_{2max}$, 3-4 days/week 2/control	1/-1.6 kg 2/+0.2 kg	↓ in IL-6 & ALT
Villareal <i>et al.</i> (2006)	6 months	27 obese older adults	2 groups: 1/diet + exercise 2/control	1/-8.2 kg 2/-0.7 kg	↓ in glucose, TAG, BP, insulin AUC, CRP & IL-6

Table 1.3: Interventions investigating the effects of exercise on metabolic control and inflammation in participants with IGT or T2D. IGT= Impaired glucose tolerance, NGT= Normal glucose tolerance, NS= Not stated, IR= Insulin resistance, BP= Blood pressure.

Author	Duration	Study population	Intervention	Body mass change	Outcome
Balducci <i>et al.</i> (2010)	1 year	606 participants with T2D	2 groups: 1/supervised exercise (150 min over 2 sessions/week) 2/counselling	↓ in BMI	↓ in HbA _{1c} , BP, LDL, IR. ↓CRP from 3mg/l to 2mg/l.
Balducci <i>et al.</i> (2010)	1 year	82 participants with T2D	4 groups: 1/control 2/ low-intensity exercise 3/high-intensity aerobic 4/ aerobic + resistance	No change in fat mass	↓ in HbA _{1c} in all exercise groups. CRP ↓ in aerobic and aerobic plus resistance (independent of changes in weight). ↓ in IL-6 and ↑ in adiponectin
Bloem <i>et al.</i> (2008)	7 days	12 sedentary older participants with IGT	Supervised exercise (1h/d of aerobic exercise).	No change in body weight	Improvements in IR. Neither CRP or Adiponectin were impacted.
Brooks <i>et al.</i> (2007)	16 weeks	62 participants with T2D	1/ strength training + standard care 2/ standard care	NS	↓ in IR, ↓ in CRP and ↑ in adiponectin
Dekker <i>et al.</i> (2007)	12 weeks	24 middle-aged men (8 lean, 8 obese, 8 obese with T2D)	performed 60 min of aerobic exercise 5 times/week	Lean: -0.4 kg Obese: -0.4 kg T2D: +0.4 kg	↓ in IL-6 in all groups, CRP wasn't affected
Jorge <i>et al.</i> (2011)	12 weeks	48 participants with T2D	4 groups (3x/week, 60 min session): 1/control 2/aerobic 3/resistance 4/combined	No change in body weight	↓ in BP, fasting and postprandial glucose, lipid profile and CRP. No difference between groups.
Kadoglou <i>et al.</i> (2007)	6 months	60 overweight participants with T2D	6-month aerobic exercise training programme (4 times/week, 45-60 min/session)	↓ in body weight (1.3 kg)	Improved glucose control, lipid profile, decreased IR. ↓ CRP, no change in adiponectin or TNF-α
Kadoglou <i>et al.</i> (2010)	1 year	100 overweight/obese participants with T2D	4 groups: 1/control 2/rosiglitazone 3/exercise 4/exercise + rosiglitazone	↓ in fat mass 1/-0.4 % 2/-0.5 % 3/ -1.6 % 4/-2.4 %	Effects of exercise and drug on glycaemic control and various inflammatory markers were additive
Marcell <i>et al.</i> (2005)	16 weeks	51 middle-aged, overweight participants with IGT	1/moderate-intensity exercise 2/ intense exercise 3/ no exercise	1/ -4.7 kg 2/ -5.6 kg 3/ -1.1 kg	Insulin sensitivity improved in the intense groups. No improvement in CRP or adiponectin
Oberbach <i>et al.</i> (2006)	4 weeks	60 participants (NGT n=20, IGT n=20, T2D n=20)	exercise program	NGT: -1.4 kg IGT: -3.2 kg T2D: -1.6 kg	Improvements in glucose metabolism and IS in IGT and T2D groups. CRP ↓ in all groups, IL-6 wasn't impacted, adiponectin ↑ in the IGT and T2D groups.

Table 1.4: Interventions investigating the effects of exercise combined with caloric restriction on metabolic control and inflammation in participants with IGT or T2D. IGT= Impaired glucose tolerance, NGT= Normal glucose tolerance, IR= Insulin resistance, BP= Blood pressure, MS=Metabolic syndrome, PA= Physical activity.

Author	Duration	Study population	Intervention	Body mass change	Outcome
Belalcazar <i>et al.</i> (2010)	1 year	1759 obese participants with T2D	2 groups: 1/ intensive lifestyle intervention 2/ usual care	1/-9.0 kg 2/-0.8 kg	Reduced adiposity, improved glucose control and fitness all seemed to partially explain changes in CRP
Bo <i>et al.</i> (2007)	1 year	335 participants with IGT	2 groups: 1/intensive lifestyle program 2/usual care	1/ -0.8 kg 2/ +1.7 kg	↓ in most of the MS components and CRP
Giannopoulou <i>et al.</i> (2005)	14 weeks	33 middle-aged women with T2D	3 groups: 1/diet alone 2/exercise alone 3/diet+ex	1/-4.6 kg 2/-1.7 kg 3/-5.4 kg	CRP ↓ by 15 % in all 3 groups. No difference in adiponectin
Haffner <i>et al.</i> (2005)	1 year	3234 participants with IGT	3 groups: 1/lifestyle intervention 2/metformin 3/placebo	1/-6.8 kg 2/-2.7 kg 3/-0.4 kg	In men, CRP ↓ by 33 % in the lifestyle arm, -7 % in metformin arm and +5 % in placebo. In women, CRP ↓ by 29 % in the lifestyle arm, -14 % in metformin arm and no change in placebo
Herder <i>et al.</i> (2009)	1 year	406 participants with IGT	2 groups: 1/intensive lifestyle program 2/usual care	1/-4.7 kg 2/-1.2 kg	↓ CRP and IL-6. Moderate to vigorous PA predicted decreases in CRP and IL-6
Lindahl <i>et al.</i> (2009)	1 month	168 participants with IGT	2 groups: 1/intensive lifestyle program 2/usual care	1/ -5.0 kg 2/ -0.7 kg	Improvements in BP, lipid profile and IR. CRP was not affected
Monzillo <i>et al.</i> (2003)	6 months	24 obese participants with IGT or T2D	combining hypocaloric diet + moderate physical activity	-6.9 kg	Significant improvements in IR and ↓ in leptin. IL-6 significantly ↓ while adiponectin ↑ significantly amongst diabetic participants
Schafer <i>et al.</i> (2007)	9 months	181 participants (133 at risk of T2D and 48 with IGT)	Lifestyle intervention	-2.8 kg	IGT was reversed in 24 out of 48 individuals, IR ↓ in both groups. Adiponectin significantly ↑ in both groups more so in the IGT, IL-6 wasn't impacted, CRP ↓ in both groups
Yokoyama <i>et al.</i> (2004)	3 weeks	40 participants with T2D	2 groups: 1/ diet alone 2/ diet + exercise	Similar small decrease in BMI in both arms	Diet + exercise improved IR further than diet alone. Adiponectin wasn't affected in either group

1.5 Metabolic control, inflammation and diet

1.5.1 Epidemiological studies

Animal studies have shown that caloric restriction slows ageing and reduces the incidence of malignancies. Such studies are difficult in free-living conditions with participants. Evidence so far seems to suggest that caloric restriction reduces risk factors for type 2 diabetes and atherosclerosis. The Comprehensive Assessment of Long-Term Effects of Reducing Calorie Intake (CALERIE) is a research program involving three research centres. Three pilot work studies examined whether investigating the impact of caloric restriction in free-living humans was feasible. Preliminary data following 6-12 months of caloric restriction (~ -25 %) in a variety of participants resulted in a 10 % reduction in body weight, fat mass and improvements in insulin sensitivity. More recent work by Redman *et al.* (2009) focussed on investigating the metabolic and behavioural adaptations to caloric restriction in free-living conditions. For the study duration of 6-months, 48 overweight participants were randomised to 4 groups: 1/ control group provided with 100 % of energy requirements, 2/ 25 % caloric restriction, 3/ 12.5 % caloric restriction plus 12.5 % increase in energy expenditure by structured exercise, 4/ low calorie diet (890 kcal/d) until 15 % weight reduction was achieved followed by weight maintenance. Similar weight loss was achieved through caloric restriction alone (-10.4 ± 0.9 %; mean \pm SE) and caloric restriction combined with structured exercise (-10.0 ± 0.8 %) while the low calorie diet led to a weight loss of -13.9 ± 0.8 %. Interestingly, the total daily energy expenditure was significantly reduced in the caloric restriction group (-454 ± 76 kcal/day) and low calorie diet group (-633 ± 66 kcal/day) but not in the control and caloric restriction and structured exercise groups. These data potentially explain why caloric restriction causes large inter-individual variability in the rates of weight loss and how lifestyle interventions combining diet and physical activity might be more successful in maintaining weight loss in the long term.

A study by Hu *et al.* (2001) followed 84 941 female nurses from 1980 to 1996. Participants were free from cardiovascular disease, type 2 diabetes

and cancer at baseline. Information about their lifestyle and diet was regularly updated. A low-risk group was defined according to five variables: BMI<25; physical exercise for at least 30 min per day; a diet high in fibre, polyunsaturated fat and low GI, non-smoker and consumption of half a drink of an alcoholic beverage per day. During the intervention, 3300 new cases of type 2 diabetes were documented with overweight/obese being the single most important predictor of developing type 2 diabetes. Smoking status, lack of physical activity, a poor diet and complete abstinence from alcohol were all associated with an increased risk of developing type 2 diabetes. Of the 3300 new cases of type 2 diabetes, 91 % of these cases could be attributed to behaviours and habits that did not conform to the low-risk pattern.

1.5.2 Dietary intervention studies and metabolic control/inflammation

Many intervention studies have attempted to investigate the effects of dietary restriction on several metabolic/inflammatory markers. Recent intervention studies suggest that lifestyle interventions have the potential to reduce the risk of developing type 2 diabetes through a targeted reduction in body weight. Improvements in metabolic and inflammatory markers seem to be increased with greater weight/fat loss following caloric restriction. The improvements are similar to those reported with increased physical activity suggesting that weight/fat loss seem to be the main contributor whether this is induced by increased physical activity, caloric restriction or a combination of both. A systematic review by Selvin *et al.* (2007) concluded that for each 1 kg of weight loss, the mean change in CRP level was -0.13 mg/L. Future studies carefully manipulating energy balance through either caloric restriction and/or increased physical activity are required to further elucidate the mechanisms by which both stimuli potentially improve metabolic function and inflammatory markers. Studies looking at the effect of diet alone including participants who are overweight/obese are summarised in Table 1.5. Dietary studies including participants who are insulin resistant or suffer from type 2 diabetes are summarised in Table 1.6. Only recent studies are presented in this section; the list is non-exhaustive.

Table 1.5: Interventions investigating the effects of diet on metabolic control and inflammation in overweight/obese participants. CR= Caloric restriction, VLCD= Very-low calorie diet, EI=Energy intake, EE= Energy expenditure, SVF= Stromavascular fraction, AT= Adipose tissue, EX= Exercise, IR= insulin resistance

Author	Duration	Study population	Intervention	Body mass change	Outcome
Clement <i>et al.</i> (2004)	4 weeks	29 obese women	2 groups: 1/28 days VLCD 2/2 days VLCD	1/-6 kg 2/-1 kg	↓ in fasting insulin, IR, TAG, cholesterol following 28 days of VLCD. Decrease of pro-inflammatory factors and increase of anti-inflammatory factors within the SVF of the AT.
Fontana <i>et al.</i> (2007)	1 year	46 middle-aged overweight participants	2 groups: 1/CR (-20 % EI) 2/Exercise (+20 % EE)	1/-8.2 kg 2/-6.6 kg	Similar reduction in fat mass and CRP between groups. Improvements in lipid profiles, HOMA-IR.
Heilbronn <i>et al.</i> (2001)	12 weeks	83 obese women	Low-fat (15 % of total energy)	-7.9 kg	Improvements in lipid profiles, significant decreased in CRP
Larson-Meyer <i>et al.</i> (2006)	6 months	48 overweight participants	4 groups: 1/control 2/25% CR 3/12.5% CR+ 12.5 % EX 4/15 % weight loss through VLCD followed by maintenance	1/+0.1 kg 2/-8.4 kg 3/-8.1 kg 4/-11 kg	Improvement in insulin in all 3 intervention groups.
Nicklas <i>et al.</i> (2004)	18 months	316 older overweight or obese sedentary men and women	4 groups: 1/ control 2/diet 3/EX 3/diet +EX	1/-2.3 kg 2/-12.8 kg 3/-4.1 kg 4/-8.2 kg	Diet-induced weight loss resulted in greater ↓ in CRP. Exercise training did not have a significant effect on the inflammatory biomarkers.
Tchernof <i>et al.</i> (2002)	~14 months	25 obese postmenopausal middle-aged women	1200 kcal/day until achieved target weight	-14.5 kg	Improvement in lipid profiles, significant decreased in CRP

Table 1.6: Interventions investigating the effects of caloric restriction on metabolic control and inflammation in participants with IGT or T2D. IGT= Impaired glucose tolerance, NGT= Normal glucose tolerance, NS= Not stated, IR= Insulin resistance, BP= Blood pressure, MS=Metabolic syndrome, PA= Physical activity, CR= Caloric restriction, VLCD= Very-low calorie diet

Author	Duration	Study population	Intervention	Body mass change	Outcome
Brinkworth <i>et al.</i> (2004)	8 weeks	66 obese participants with T2D	2 groups: 1/ low-protein diet 2/ high-protein diet 8 weeks of CR followed by 4 weeks of energy balance. Maintained diet for 12 months	1/-2.2 kg 2/-3.7 kg Data at week 64	38 participants completed study. ↑ in HDL cholesterol ↓ in CRP
Esposito <i>et al.</i> (2004)	2 years	180 participants (99 men/81 women) with MS	2 groups: 1/ an intervention group (Mediterranean diet) 2/control group (prudent diet)	1/-4 kg 2/-1.2 kg	Participant in the intervention group significantly ↓ their CRP, IL-6 and IR levels compared to the control group
Hammer <i>et al.</i> (2008)	16 weeks	12 obese, insulin treated participants with T2D	VLCD, insulin was stopped during the study	Δ in average BMI from 35.6 to 27.5	Improvements in HbA _{1c} , Adiponectin significantly increased (Δ2.6ug/l) and CRP was drastically reduced (Δ11mg/l)
Jenkins <i>et al.</i> (2008)	6 months	210 participants with type 2 diabetes treated with anti-hyperglycaemic medications	2 groups: 1/high-cereal fibre diet 2/low-glycaemic index dietary advice.	1/-1.6 kg 2/-2.5 kg	In patients with type 2 diabetes, 6-month treatment with a low-glycaemic index diet resulted in moderately lower HbA _{1c} levels compared with a high-cereal fibre diet.
Petersen <i>et al.</i> (2005)	3-12 weeks	8 obese participants with T2D	liquid diet formula which was supplemented with fruits and vegetables to ~1200 kcal/d until achievement of normo-glycaemia (3-12 weeks) followed by 4 weeks of weight stabilisation	8 kg	Fasting hyperglycaemia was normalised. Neither IL-6 or Adiponectin were impacted by the intervention.
Wolever <i>et al.</i> (2008)	1 year	162 participants with T2D	3 groups: 1/high CHO, high GI diet 2/high CHO, low GI diet 3/low CHO, high monosaturated fat diet	Fell over the first 8 weeks then rose steadily	CRP decreased similarly in the high GI and low GI diet but increased in the low CHO diet group. CRP values were significantly different between groups at baseline.
Xydakis <i>et al.</i> (2004)	4-6 weeks	80 men and women, 40 with metabolic syndrome	VLCD (600-800 kcal/d) 2 groups: 1/with MS 2/without MS	1/-8.1 kg 2/NS	CRP ↓ in participants with MS. Adiponectin was not affected.

1.5.3 Potential mechanisms for independent benefits of physical activity

Although it appears that regular physical activity is effective in preventing several chronic diseases (e.g., hypertension, cardiovascular disease, diabetes and obesity) it is still unclear whether physical activity *per se* improves markers of inflammation and metabolic health. Several biological mechanisms may contribute to the reduction in the risk of developing chronic diseases. Regular physical activity has been shown to improve body composition and improve lipid profiles (Warburton *et al.*, 2001). It is possible that one of the mechanisms by which regular exercise impacts health is by maintaining a metabolically healthy adipose tissue. A positive energy balance will ultimately result in an increase in visceral and subcutaneous adipose tissue followed by the infiltration of pro-inflammatory T cells and macrophages leading to chronic low-grade systemic inflammation (Gleeson *et al.*, 2011).

A series of changes take place as a result of regular physical activity including a turnover of liver and skeletal muscle glycogen stores. This results in an increase in glycogen synthase (Christ-Roberts *et al.*, 2004), hexokinase activity (Mandroukas *et al.*, 1984) and GLUT4 mRNA and protein expression (Dela *et al.*, 1994). This regular turnover might be one of the mechanisms by which regular physical activity benefits health.

Pedersen (2007) suggest that the increase in IL-6 released by contracting muscle during exercise results in a net anti-inflammatory effect. This is followed by an increase in anti-inflammatory cytokines such as IL-10. Therefore, it is possible that the anti-inflammatory effects of regular exercise could prevent an increase in chronic low-grade inflammation.

1.6 Aim

This review has demonstrated that reduced physical activity and excess weight/fat resulting from long term positive energy balance are associated with increased concentrations of inflammatory markers as well as a reduction in insulin sensitivity. This pro-inflammatory environment and increased concentrations of these markers are associated with increased risks of developing chronic diseases such as type 2 diabetes, the metabolic syndrome and cardiovascular diseases. A reduction in weight/fat mass through an energy deficit appears to be associated with improvements in markers of inflammation and metabolic control. Whether this is best achieved via caloric restriction, increased physical activity or a combination remains unclear. Therefore, the aim of this thesis is to investigate the impact of physical activity/exercise and energy balance on metabolic control and inflammation and whether the potential benefits of exercise are specific to its characteristics (such as duration and intensity)

CHAPTER 2

General Methods

2.1 Introduction

This chapter outlines the generic methodologies that were used throughout the Chapters 4 and 5 of this thesis. The methods in Chapter 3 were specific and are described therein. All human experimental investigations were conducted within the Applied Physiology Research Laboratory at the University of Bath. Each study received ethical approval from the Bath NHS Research Ethics Committee prior to commencement.

In Chapter 4, subjects were habitually active males aged 18-40 years. Participants described in Chapter 5 were sedentary, overweight/obese (body mass index (BMI) $>25 \text{ kg}\cdot\text{m}^{-2}$) males and postmenopausal females aged 45-64 years. Volunteers were recruited via advertisement within the local community. Prior to taking part in any study, participants were provided with an information sheet detailing the requirements of the research. All subjects gave written consent to participate and were asked to complete a medical history questionnaire (Appendices 1 & 2) and a Physical Activity Readiness Questionnaire (PAR-Q; Appendix 3). Individuals who smoked, suffered from a condition known to interact with the study measures or took regular medication that may have interfered with the results were excluded from the study.

2.2 Anthropometry

Height was measured and recorded to the nearest 0.1 cm using a fixed stadiometer (Holtain Ltd., UK). Body mass was measured and lean and fat mass estimated and recorded to the nearest 0.1 kg using biological impedance enabled electronic scales (Tanita Corporation, Japan). All subjects were required to wear the same light clothing for this measurement on every occasion. BMI was calculated for all subjects as $\text{BMI} = \text{weight (kg)} / \text{height (m}^2\text{)}$. Fat Mass Index (FMI) was calculated for all subjects as $\text{FMI} = \text{fat mass (kg)} / \text{height (m}^2\text{)}$. Waist and hips circumference were measured in triplicate using a metallic tape measure (Lufkin, US); the mean value was calculated. Lean and fat mass were also

estimated using Dual-energy X-ray absorptiometry (DEXA). After descriptive details were entered into the QDR for Windows software (Hologic, Bedford, UK), subjects were asked to lie supine on the DEXA scanning table (Discovery, Hologic, Bedford, UK). Participants were positioned centrally on the scanning table with feet spaced evenly either side of the mid-point of the body with arms placed mid-prone with an equal gap to the trunk on both sides. Participants were requested to remain still whilst the scan was performed by an individual trained in ionising radiation regulations (medical exposure). Scans were analysed for total fat mass, abdominal fat mass (L1-L4), lean mass, bone mineral content and fat percentage following the guidelines described in the QDR for Windows manual (Hologic, Bedford, UK).

2.3 Blood Pressure

Blood pressure was measured using an automated blood pressure monitor (Dinamap PRO Series 100, GE Medical Systems, Germany) in a seated position after subjects had rested for at least 10 minutes. Three readings were taken; the mean value was calculated.

2.4 Blood Sampling

All participants reported to the laboratory in the morning after an overnight fast (no food or drink except water for at least 10 hours). Following the application of a topical local anaesthetic (1.5 ml Ametop gel, Smith & Nephew, Hull, England) an 18-gauge 1.3 x 45 mm cannula (BD Venflon Pro) was inserted into an antecubital vein. Participants remained in a supine position for 15 min prior to all blood sampling. Venous blood samples were obtained using a stopcock and syringe at several time points throughout the trial. The cannula was kept patent by periodic flushing with 0.9 % Sodium Chloride solution (B.Braun, UK). All blood samples were collected using syringes. For the Oral Glucose Tolerance Test (OGTT), participants were required to drink 113 ml (75 g) of a glucose (maltodextrin) solution (Polycal, Nutricia, UK) within 5 min. Blood samples were collected every 15 min for 2 hours. A 5 ml waste sample was drawn and discarded before collecting each sample. Blood was dispensed into collection tubes (Sarstedt Ltd., Leicester, UK) containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant for plasma samples or serum-separator tubes for serum samples.

2.5 Blood Analysis

Whole blood differential leukocytes counts were obtained using an automated haematology system (SF-300, Sysmex Ltd., Milton Keynes, UK). Plasma tubes were centrifuged straight away (Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., England) at 5000 rpm (3500 g) for 10 min at 4°C. Serum samples were left to clot for 15 minutes at room temperature before being centrifuged (Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., England) at 5000 rpm (3500 g) for 10 min at 4°C. Serum and plasma were transferred to separate tubes before being stored at -80°C. All blood analyses were performed in duplicate. Immunoassays for serum triacylglycerol (TAG), total and high-density lipoprotein (HDL) cholesterol, alanine transaminase (ALT), plasma glucose (Randox, Crumlin, Co. Antrim, UK), non-esterified fatty acids (NEFA; Wakochemicals GmbH, Germany) were performed using a Cobas Mira (Cobas, Roche Diagnostics Limited, UK). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (Friedewald *et al.*, 1972). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting glucose ($\text{mmol}\cdot\text{l}^{-1}$) x fasting insulin ($\text{mU}\cdot\text{l}^{-1}$) / 22.5 (Turner *et al.*, 1979). Homeostasis model assessment for β -cell function (HOMA- β) was calculated as fasting insulin ($\text{mU}\cdot\text{l}^{-1}$) x 20 / fasting glucose ($\text{mmol}\cdot\text{l}^{-1}$) - 3.5 (Matthews *et al.*, 1985). The Insulin Sensitivity Index (ISI comp/Matsuda Index) was calculated as $10000 / \text{square root}(\text{fasting glucose}(\text{mg}\cdot\text{dl}^{-1}) \times \text{fasting insulin}(\mu\text{U}\cdot\text{ml}^{-1}) \times (\text{mean OGTT glucose value} \times \text{mean OGTT insulin value}))$ (Matsuda & DeFronzo, 1999).

Commercially available enzyme-linked immunosorbent assays (ELISA) were used to measure serum Adiponectin, Leptin, C-Reactive Protein (CRP) (Quantikine, R&D Systems Inc., Abingdon, UK) Interleukin 6 (IL-6; Quantikine HS, R&D Systems Inc., Abingdon, UK) Insulin, C-peptide (Mercodia AB, Uppsala, Sweden). Absorption was determined using a microplate reader (HTIII, Anthos Labtec Instruments Ltd., Ringer, East Sussex) at the wavelengths specified by the kit manufacturer. Coefficients of variation and minimal detectable concentrations provided by the manufacturer for each ELISA are summarised in Table 2.1.

Table 2.1: Reported sensitivity, intra and inter-assay precision for all ELISAs used.

Parameter	Sensitivity	Intra-assay precision (CV)	Inter-assay precision (CV)
Adiponectin	0.25 ng·ml ⁻¹	3.5 %	6.5 %
Leptin	7.8 pg·ml ⁻¹	3.2 %	4.4 %
CRP	0.01 ng·ml ⁻¹	5.5 %	6.5 %
hs IL-6	0.04 pg·ml ⁻¹	7.4 %	7.8 %
Insulin	1 mU·l ⁻¹	3.4 %	3.0 %
C-peptide	15 pmol·l ⁻¹	3.6 %	3.3 %

2.6 Sub-maximal and Maximal Oxygen Uptake ($\dot{V}O_{2\max}$) Testing

In Chapter 4, participants' preliminary visit to the laboratory comprised two exercise tests following a method adapted from Taylor *et al.* (1955). The first of these tests determined the oxygen uptake ($\dot{V}O_2$) of each participant at a variety of sub-maximal running speeds on a treadmill ergometer (Woodway, ELG 70, Weiss, Germany). Typically this test was completed within 20 min, with the treadmill speed increasing at 5 min intervals such that a range of at least 4 intensities could be monitored. For most participants, treadmill speeds of 10,11,12 and 13 km·h⁻¹ were sufficient to encompass all the relative exercise intensities employed during subsequent exercise sessions (nominally spanning a heart rate of 170 beats·min⁻¹). During the final minute of each 5 min stage, expired gas samples along with ratings of perceived exertion (RPE; Borg, 1973) and heart rates (HR) were recorded. HR were recorded throughout all these investigations via short range telemetry (Polar RS200, Finland). Gas samples were taken at the end of every stage to determine the relationships between $\dot{V}O_2$, exercise intensity (speed) and HR in order to calculate the correct speed required to obtain 60 % and 70 % $\dot{V}O_{2\max}$.

Approximately 20 min following the sub-maximal oxygen uptake test, participants completed a second test to assess their $\dot{V}O_{2\max}$. This test involved incremental continuous treadmill running until the point of volitional exhaustion. The speed at which this test was conducted was dictated by the data recorded during the sub-maximal test with a speed initially eliciting approximately 75-85 % of age-estimated maximum HR was selected. Participants ran at this speed

against a 2.5 % gradient which was increased by 2.5 % after every 3 min stage. One minute expired air samples, RPE and HR were measured in the final minute of each stage and also at the point of volitional exhaustion, defined as when the participant indicated that only 1 min remained until fatigue. A number of criteria were applied to determine whether this endpoint was reflective of a valid $\dot{V}O_{2\max}$ value, these were: attainment of age predicted maximal HR (± 10 beats min^{-1}); a respiratory exchange ratio (RER) in excess of 1.15; an increase in $\dot{V}O_2 \leq 5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in response to an increased gradient; and an RPE = 20. Each participant met at least two of these four criteria. While progressive incline protocols may be less likely to fulfil all 4 of these criteria in comparison with level running protocols, the resultant $\dot{V}O_{2\max}$ value is not thought to differ significantly between the two methods (St. Clair Gibson *et al.*, 1999).

In Chapter 5, participants underwent a graded walking stress test according to a modified Bruce protocol to assess their $\dot{V}O_{2\max}$. This test involved incremental continuous treadmill walking until the point of volitional exhaustion. For most participants, a treadmill speed of $6 \text{ km} \cdot \text{h}^{-1}$ was chosen. Participants walked at this speed against a 0 % gradient which was increased by 2.5 % after every 3 min stage. One minute expired air samples, RPE and HR were measured in the final minute of each stage and also at the point of volitional exhaustion, defined as when the participant indicated that only 1 min remained until fatigue. The same criteria described above were applied to determine whether this endpoint was reflective of a valid $\dot{V}O_{2\max}$ value. Gas samples were taken at the end of every stage to determine the relationships between $\dot{V}O_2$, exercise intensity (gradient) and HR in order to calculate the correct gradient and speed required to obtain either 50 % or 70 % $\dot{V}O_{2\max}$.

2.7 Expired Gas Analysis

Expired gases were collected into Douglas bags with participants wearing a respiratory valve (mouthpiece) and nose clip throughout gas collection. The respiratory valve was worn for at least 30 s before gas sample collection in order to flush the respiratory valve and associated tubing of atmospheric air and settle any initial hyperventilation. The percentage of oxygen (O_2) and carbon dioxide (CO_2) in expired air samples was determined using paramagnetic and infrared gas analysers, respectively (Series 1400, Servomex Ltd., Sussex, UK). These

analysers were calibrated within 1 hour of each test with gases of known composition within the physiological range (British Oxygen Company, UK). Expired air volume was determined using a dry gas meter (Harvard Apparatus, Kent, UK) and corrected to standard temperature and pressure (dry gas). The total volume of expired air was taken as the sum of the volume used for gas composition analysis and that evacuated by the dry gas meter. Room temperature and pressure were recorded. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were calculated for each expired air sample as determined by the Haldane transformation. RER was calculated ($\dot{V}CO_2 / \dot{V}O_2$) for each expired air sample.

2.8 Assessment of Physical Activity

Habitual physical activity was determined using a physical activity monitor (Actiheart, Cambridge Neurotechnology Ltd., Cambridge, UK). This is a combined heart rate monitor and accelerometer. The monitor was worn for seven whole, consecutive days, recording data every minute over the time period worn. Participants were not informed of the function of the monitor so as to minimise changes in behaviour. This data was then downloaded and a software program (Actiheart 2.0, Cambridge Neurotechnology Ltd., Cambridge, UK) was used to produce a plot of the heart rate and accelerometry data (Figure 2.1), as well as an approximated Metabolic Equivalent of Task (MET) level at each time point, calculated from these two variables. The software was used to estimate Activity Energy Expenditure (AEE) (Figure 2.2) using branched-equation modelling, providing precise estimates of energy expenditure in minute-by-minute epochs validated by Brage *et al.* (2005) and Thompson *et al.* (2006).

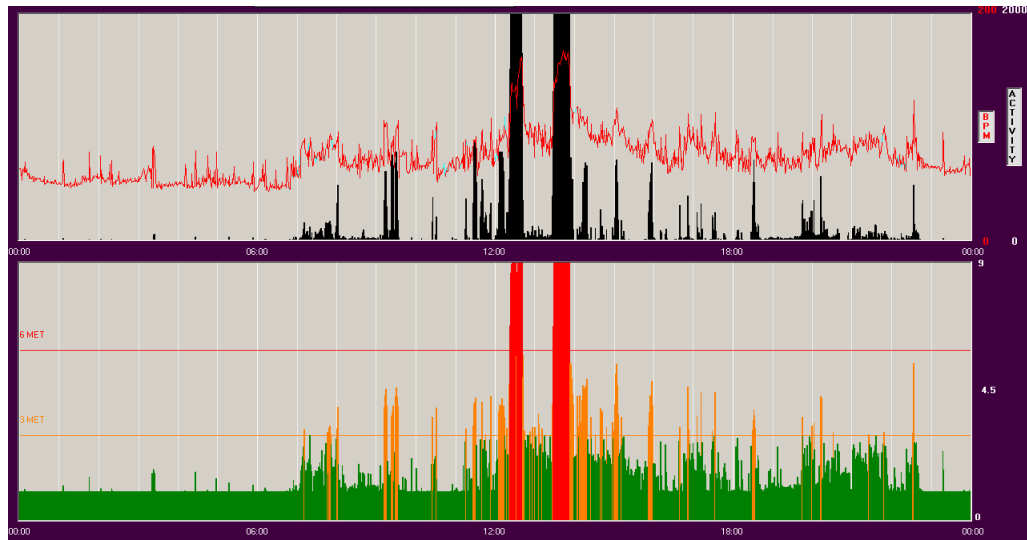


Figure 2.1: Example of an output from the Actiheart software. The top plot shows the activity ($\text{counts}\cdot\text{min}^{-1}$) and heart rate data ($\text{beats}\cdot\text{min}^{-1}$), against time, over a 24 h period. The bottom plot shows number of METS against time.

		0	2000	4000	REE	AEE	DIT	TEE	PAL
Thu	14/10/2010				501	215	80	795	
Fri	15/10/2010				1639	1014	295	2948	1.80
Sat	16/10/2010				1639	1115	306	3060	1.87
Sun	17/10/2010				1639	893	281	2814	1.72
Mon	18/10/2010				1639	720	262	2622	1.60
Tue	19/10/2010				1639	799	271	2709	1.65
Wed	20/10/2010				1639	1497	349	3485	2.13
Thu	21/10/2010				1639	559	244	2443	1.49
Fri	22/10/2010				911	215	125	1251	
Average of full days					1639	942	287	2869	1.75

Figure 2.2: Example of an output from the Actiheart software. The figure shows the estimated Resting Energy Expenditure (REE), Activity Energy Expenditure (AEE), Diet Induced Thermogenesis (DIT), Total Energy Expenditure (TEE) and Physical Activity Level (PAL; TEE/REE) as well as the average for the seven days during which the Actiheart was worn.

2.9 Assessment of Dietary Intake

Participants were asked to record their food and fluid intake for a “typical” whole week (7 days) prior to taking part in the study. Each subject received a set of weighing scales (Model 3001, Salter, Kent, UK) to accurately fulfil this requirement. Subjects were asked to consume their habitual diet and not to eat less than usual during the recorded week. Diets were analysed using the software CompEat Pro Version 5.8.0 (Nutrition Systems, UK), which is based on food composition tables for UK foods. Energy intake was estimated using the software and DIT was estimated pre-intervention as 10 % of energy intake (Westerterp, 2004).

2.10 Adipose Tissue Biopsy

After cleaning the skin with an Iodine-based solution (Videne Alcoholic Tincture, Ecolab, UK), a subcutaneous adipose tissue sample was obtained from the abdominal wall, 4-7 cm lateral of the umbilicus. Once the Iodine-based solution had dried, ~5 ml of a 1 % Lidocaine Hydrochloride solution (Hameln Pharmaceuticals Ltd., Gloucester, UK) was injected using a 27 G needle in a fan-like pattern into the biopsy site. After waiting 5 min for the area to become anaesthetised, a 50 ml syringe was filled with 5-10 ml of a 0.9 % Sodium Chloride solution (B.Braun, UK) and fitted with a 14 G needle. The needle was inserted through the skin and into the fat. Suction was applied by pulling the plunger of the 50 ml syringe out; a piece of rigid plastic was then inserted between the end of the barrel and the end of the plunger to create a vacuum in the syringe. Adipose tissue was obtained by rocking the needle back and forth until enough tissue was obtained. After removing the needle, pressure was applied for at least 15 min until bleeding had stopped. Follow-up biopsies were taken from the opposite side.

2.11 Adipose Tissue Sample Processing

The sample was cleaned with isotonic saline and any clot was manually removed. After weighing the sample, it was homogenised in 5 ml of Trizol (Invitrogen, UK) and stored at -80°C. Samples were defrosted and spun for 5 min at 2500 g, 4°C. The top layer and pellet were removed. 200 µl of Chloroform was added per 1ml of Trizol. After shaking the mixture vigorously for 15 s, samples were incubated at room temperature for 3 min and then spun for 5 min at 2500 g, 4°C.

The aqueous phase was removed and used for gene expression analysis (Chapter 2.12) while the organic phase was used for Western Blot analysis (Chapter 4.2.7).

2.12 Gene Expression Analysis

The aqueous phase was mixed with an equal volume of 70 % Ethanol before being loaded on an RNeasy mini column (Qiagen, Crawley, UK). Total RNA was then extracted using the standard protocol supplied with the columns. Each sample was quantified using spectrophotometry. 2 µg of total RNA was then reverse transcribed using a high capacity cDNA Reverse Transcription kit (Applied Biosystems, Warrington, UK). Pre-designed primers and probes were obtained from Applied Biosystems for each gene tested. Real-time PCR was performed using a StepOneTM (Applied Biosystems, Warrington, UK). Peptidylpropyl isomerase A (PPIA) was used as an endogenous control. The comparative Ct method was used to process the data where $\Delta Ct = Ct \text{ Target gene} - Ct \text{ Endogenous control}$ and where $\Delta\Delta Ct = \Delta Ct \text{ Sample} - \Delta Ct \text{ Reference}$. Fold changes were calculated using the following formula; $\text{Fold change} = 2^{-\Delta\Delta Ct}$. The average fold change was calculated for each group.

CHAPTER 3

Diet or diet plus physical activity provide similar positive changes in inflammation in patients with newly-diagnosed type 2 diabetes compared to usual care: The Early ACTID randomised controlled trial

3.1 Introduction

Epidemiological studies indicate that a variety of systemic markers of low-grade inflammation are related to risk of future cardiovascular events in people with diabetes (Stehouwer *et al.*, 2002; Pearson *et al.*, 2003; Ridker, 2003; Rosenson & Koenig, 2003; Schillinger *et al.*, 2003; Rutter *et al.*, 2004; Schulze *et al.*, 2004; Kanai *et al.*, 2008). Of the measures that are readily available, high sensitivity serum concentrations of C-reactive protein (CRP) is probably the most widely used and appears to have consistent predictive value. In patients with Type 2 Diabetes, CRP is an independent risk factor for cardiovascular events (Schillinger *et al.*, 2003; Rutter *et al.*, 2004; Schulze *et al.*, 2004), risk of death (Stehouwer *et al.*, 2002), atherosclerotic progression (Kang *et al.*, 2004) and development of peripheral vascular disease (Yu *et al.*, 2004). Circulating CRP concentrations increase continuously across the spectrum of fasting glucose concentrations beginning at the lowest quartile of normal fasting glucose (Aronson *et al.*, 2004).

Measures of inflammation capture additional information to conventional cardiovascular risk factors (Biondi-Zoccai *et al.*, 2003; Sattar *et al.*, 2003; Ridker, Wilson & Grundy, 2004) and therefore represent an important adjunct to existing procedures (Pearson *et al.*, 2003). Since it appears unlikely that CRP per se actually *causes* cardiovascular disease (Wensley *et al.*, 2011), it has been argued that other inflammatory molecules are more likely to represent direct mediators and thus should also be measured (Brunner *et al.*, 2008; Wensley *et al.*, 2011). In this context, it is noteworthy that increased hepatic secretion of CRP is generally thought to be triggered by an increase in systemic Interleukin-6 (IL-6; Heinrich, Castell & Andus, 1990). Since IL-6 is pro-inflammatory and adipose tissue is a particularly important source of IL-6 (Mohamed-Ali *et al.*, 1997), it is possible that IL-6 represents a mechanistic link between adiposity or

adipose function and hepatic secretion of CRP (i.e., with IL-6 playing a direct pro-inflammatory role and CRP acting as an excellent surrogate marker partly by virtue of considerations such as a relatively long half-life (Vigushin *et al.*, 1993). Increased adiposity is also associated with lower serum concentrations of adiponectin (Rajala & Scherer, 2003) and this adipokine has direct anti-inflammatory effects (Rajala & Scherer, 2003; Pischon *et al.*, 2004; Shimada, Miyazaki & Daida, 2004; Schulze *et al.*, 2004). Thus, measurement of IL-6 and adiponectin provide information on pro- and anti-inflammatory pathways, respectively, and these may be heavily influenced by adipose tissue accumulation and dysfunction. In contrast, other markers of inflammation such as soluble adhesion molecules have a non-adipose origin being derived primarily from endothelial cells and leukocytes. Such markers provide information on inflammation within the vasculature (Pradhan, Rifai & Ridker, 2002; Blankenberg, Barbaux & Tiret, 2003) and it is noteworthy that soluble Intercellular Adhesion Molecule-1 (sICAM-1) independently predicts the risk of cardiovascular disease (Shai *et al.*, 2006) and the risk of stroke in people with Type 2 Diabetes (Kanai *et al.*, 2008). Thus, the measurement of multiple measures of inflammation will provide more information about these complex inter-related biological processes than a single measure alone.

Increased physical activity and dietary advice implemented by healthcare professionals is recommended by international consensus in the management of type 2 diabetes (Nathan *et al.*, 2009). The impact of diet and/or physical activity on markers of inflammation in patients with type 2 diabetes has been poorly characterised. Although there is reasonable evidence that weight loss alone improves inflammatory markers in patients with type 2 diabetes (Hotta *et al.*, 2000; Brinkworth *et al.*, 2004; Hammer *et al.*, 2008), the evidence base to demonstrate whether physical activity has the potential to elicit changes in chronic inflammation over and above those gained through weight loss alone is weak. Several studies confirm that intensive interventions designed to reduce weight and/or increased participation in supervised exercise can lead to profound beneficial effects on inflammatory markers in people with type 2 diabetes (e.g., Xydakis *et al.*, 2004; Jorge *et al.*, 2011; Kadoglou *et al.*, 2013). One larger study confirmed that supervised exercise can reduce CRP in people with type 2 diabetes (Balducci *et al.*, 2010). It is critical to answer the central question of

whether physical activity has relative benefits in established diabetes over and above those induced by dietary restriction alone in order to better inform clinical practice and patient guidelines.

The aim of this study is to investigate the impact of diet and diet plus physical activity on inflammation in the Early ACTID randomized controlled trial (Andrews *et al.*, 2011). Early ACTID is a large study that was designed to evaluate the impact of physical activity over-and-above the impact of dietary intervention whilst controlling for overall contact time with patients. The physical activity was unsupervised and community-based in order to increase the potential relevance of the findings for clinical care given that the provision of supervised physical activity will be unlikely in most healthcare settings because of resources that would be required.

3.2 Methods

3.2.1 Context

Early ACTID was a multicentre, parallel-group, randomized controlled trial. The study was approved by the Bath Research Ethics Committee (05/Q2001/5), and all participants provided written informed consent. This study is registered (number ISRCTN92162869) and has been described in detail previously (Andrews *et al.*, 2011). Early ACTID is a large study and represents the work of many investigators. The unique results reported in this thesis represent the biochemical and statistical analysis of four inflammatory outcomes (CRP, IL-6, sICAM-1, and adiponectin) at each time point during Early ACTID (approximately 600 unique samples and thus ~1800 measurements in total for each analyte).

3.2.2 Patients

Briefly, eligible patients had been diagnosed within the previous 5–8 months and were older than 30 years at diagnosis. Exclusion criteria were: age older than 80 years, HbA_{1c} concentration greater than 10 %, blood pressure higher than 180/100 mmHg, LDL cholesterol concentration higher than 4 mmol l⁻¹, body-mass index (BMI) lower than 25 kg m⁻², mass greater than 180 kg, use of weight-loss drugs, taking a sulphonylurea at the maximum dose, unstable angina, a

myocardial infarction within the previous 3 months, inability to increase physical activity, and pregnancy or planning to become pregnant.

3.2.3 Randomisation

As described previously, patients were assigned using a computer-generated allocation in a 2:5:5 ratio to usual care, an intensive diet intervention, or the intensive diet intervention plus activity (Andrews *et al.*, 2011). Allocation was stratified by centre and minimised by age, sex, fitness, route into the study and blood pressure.

3.2.4 Procedures

The usual care arm was designed to serve as a control group and consisted of standard dietary and exercise advice after randomisation, with reviews by a study doctor and nurse at baseline, 6 and 12 months.

The intensive diet intervention arm aimed to enable patients to lose and maintain a 5-10 % reduction of their initial body weight (Andrews *et al.*, 2011). The diet was not prescriptive; goals were negotiated individually with each participant. Participants saw a dietician at 3, 6, 9, and 12 months and this was supplemented by dietary advice and goal setting by nine 30 min appointments with study nurses; approximately one every 6 weeks over the course of the study.

Patients in the intensive diet and physical activity group received the same dietary intervention as the intensive diet group but were also asked to undertake at least 30 min brisk walking on at least 5 days per week over-and-above their existing physical activity. Each patient was given a pedometer (Digiwalker CW200, Yamax, Japan) and a folder containing motivating literature and pages for recording daily physical activity (pedometer readings). Activity targets were gradually increased over 5 weeks and maintained for the remainder of the study. Activity was discussed during the same nurse appointments in order to keep the total contact time the same as in the intensive diet intervention group.

3.2.5 Clinical management of patients

Management of type 2 diabetes, blood pressure, and lipid profile was undertaken by the study team for the period of the trial. Any changes in treatment of these

features were made by a doctor unaware of treatment allocation and according to a strict trial protocol to keep the risk of performance bias to a minimum. Diabetes treatment was only changed in the first 6 months in exceptional cases whereas, during the second 6 months, patients were treated to achieve pre-determined clinically-relevant targets.

3.2.6 Inflammatory Markers

Serum C-reactive protein (CRP) was determined using an automated high sensitivity immunoturbidimetric assay and RX Daytona clinical chemistry analyser (Randox Laboratories Ltd., UK). Serum was analysed for IL-6 (Quantikine HS, R&D Systems Inc., Abingdon; UK), sICAM-1 and Adiponectin using commercially-available solid phase ELISAs (Quantikine, R&D Systems Inc., Abingdon; UK). Average intra- and inter-assay coefficient of variation (CV) was established from the repeated analysis of 20-60 samples at different concentrations.

3.2.7 Statistical analysis

The primary endpoint was the difference between arms in the change in CRP from baseline to 6-months, adjusting in an ANCOVA model for the minimisation variables (age, sex, fitness, route into the study, blood pressure), the baseline value, and study centre. Importantly, in the first 6 months of Early ACTID medication was not adjusted. Planned comparisons were conducted between diet plus activity vs. usual care, diet vs. usual care, and diet plus activity vs. diet, with no adjustment for multiple comparisons (Perneger, 1998). The same analysis was conducted for the secondary outcome variables; IL-6, s-ICAM-1, and Adiponectin, and repeated for the 12-months timepoint for all variables. Mean effects are presented together with their 95 % confidence intervals. Outcome variables were natural log-transformed prior to analysis, to normalise residuals and stabilise variance. Resulting effects are therefore presented as percent (ratio) differences between arms in change from baseline, derived from back-transformation of the differences on the log scale. Analysis was according to intention-to-treat using a full information maximum likelihood method (Enders & Bandalos, 2001) using the Stata (version 12.1, Stata Corp, College Station, Texas, USA) structural equation modelling (SEM) module. Both CRP and IL-6 can be influenced by acute infections and so we used a sensitivity analysis to

explore whether excluding high values ($>10 \text{ mg l}^{-1}$ or 20 mg l^{-1} for CRP and $>10 \text{ pg ml}^{-1}$ for IL-6) influenced the outcome. Excluding samples with values above these thresholds made no material difference to the results and so the data for all available samples is presented. For the 6-month timepoint, we examined the extent (expressed as a percentage) to which the total observed effects were mediated by three putative mechanism variables – change in body mass, change in HbA_{1c} , and change in HOMA-IR. This mediation analysis was also conducted using the Stata SEM mediation module. The Pearson rank correlation was employed to determine the strength of relationships between parameters. As part of this collaborative project, the adjusted ANCOVA element of the statistical analysis was conducted by a statistician (Professor Alan Batterham, Teesside).

3.3 Results

3.3.1 Baseline

Of 1634 patients who were screened by telephone, 593 were enrolled and the characteristics of each group were similar at baseline (Table 3.1). Approximately 90 % of potential samples were available for the assessment of inflammatory markers (Figure 3.1). As reported in detail previously (Andrews *et al.*, 2011), HbA_{1c} was reduced similarly in both intervention groups compared to usual care with no difference between intervention arms. Briefly, HbA_{1c} was reduced by -0.28 % (-0.46 to -0.10) and -0.26 % (-0.44 to -0.08) in the diet group compared to usual care at 6 and 12 months, respectively. This was similar to the reduction seen in the diet plus physical activity group of -0.33 % (-0.51 to -0.14) and -0.21 % (-0.39 to -0.02) at 6 and 12 months. Measures of insulin resistance followed a similar pattern to the changes in HbA_{1c} with both intervention arms being different to usual care but not to each other. Log HOMA-IR was reduced by -0.26 (-0.37 to -0.15) and -0.24 (-0.37 to -0.12) in the diet group compared to usual care at 6 and 12 months, respectively. This was not different to the reduction seen in the diet plus physical activity group compared to usual care of -0.24 (-0.35 to -0.13) and -0.16 (-0.29 to -0.04) at 6 and 12 months. The change in weight was not different between intervention arms but both diet and diet plus physical activity groups were different to usual care at 6 and 12 months. Weight was reduced by -2.70 kg (-3.94 to -1.47) and -2.80 kg (-4.05 to -1.55) and -2.31

kg (-3.55 to -1.07) and -2.54 kg (-3.80 to -1.28) in diet and diet plus physical activity versus usual care at 6 and 12 months, respectively. The diet plus physical activity group increased their step count from 6399 (SD 3056) to 7680 (SD 2818) and 7621 (SD 2778) at 6 and 12 months, respectively.

Table 3.1: Summary statistics at baseline. Unless otherwise indicated, values represent means (SD). The mean shown is the back-transformed mean of the log transform, and the dispersion is the SD expressed as a factor (\times/\div by).

	Diet (n=248)	Diet plus activity (n=246)	Usual Care (n=99)
Male sex (%)	158 (64 %)	165 (66 %)	62 (63 %)
Age (years)	60 (10)	60 (10)	60 (11)
Smoker	24 (10 %)	16 (7 %)	8 (8 %)
White	239 (96 %)	232 (94 %)	96 (97 %)
Median (IQR) time since diagnosis (days)	186 (152-225)	194 (151-233)	185 (148-232)
Weight (kg)	90.2 (16.7)	91.1 (16.9)	93.9 (19.0)
BMI (kg m ⁻²)	31.5 (5.7)	31.6 (5.6)	32.3 (5.9)
Diabetes medication (%)	98 (40 %)	95 (39 %)	35 (35 %)
Antihypertensive agents (%)	168 (68 %)	139 (57 %)	58 (59 %)
Lipid-lowering drugs (%)	162 (65 %)	150 (61 %)	63 (64 %)
HbA _{1c} (%)	6.64 (0.93)	6.69 (0.99)	6.72 (1.02)
Systolic blood pressure (mmHg)	133 (15)	133 (15)	135 (14)
Diastolic blood pressure (mmHg)	79 (8)	79 (8)	80 (9)
Log HOMA-IR	1.60 (0.74)	1.65 (0.91)	1.62 (0.57)
CRP (mg l ⁻¹)	2.0 (3.0)	2.0 (2.9)	2.4 (3.4)
IL-6 (pg ml ⁻¹)	2.0 (1.9)	2.0 (1.8)	2.4 (1.9)
sICAM-1 (ng ml ⁻¹)	249 (1.3)	242 (1.3)	255 (1.3)
Adiponectin (μg ml ⁻¹)	5.0 (1.7)	4.8 (1.7)	5.0 (1.7)

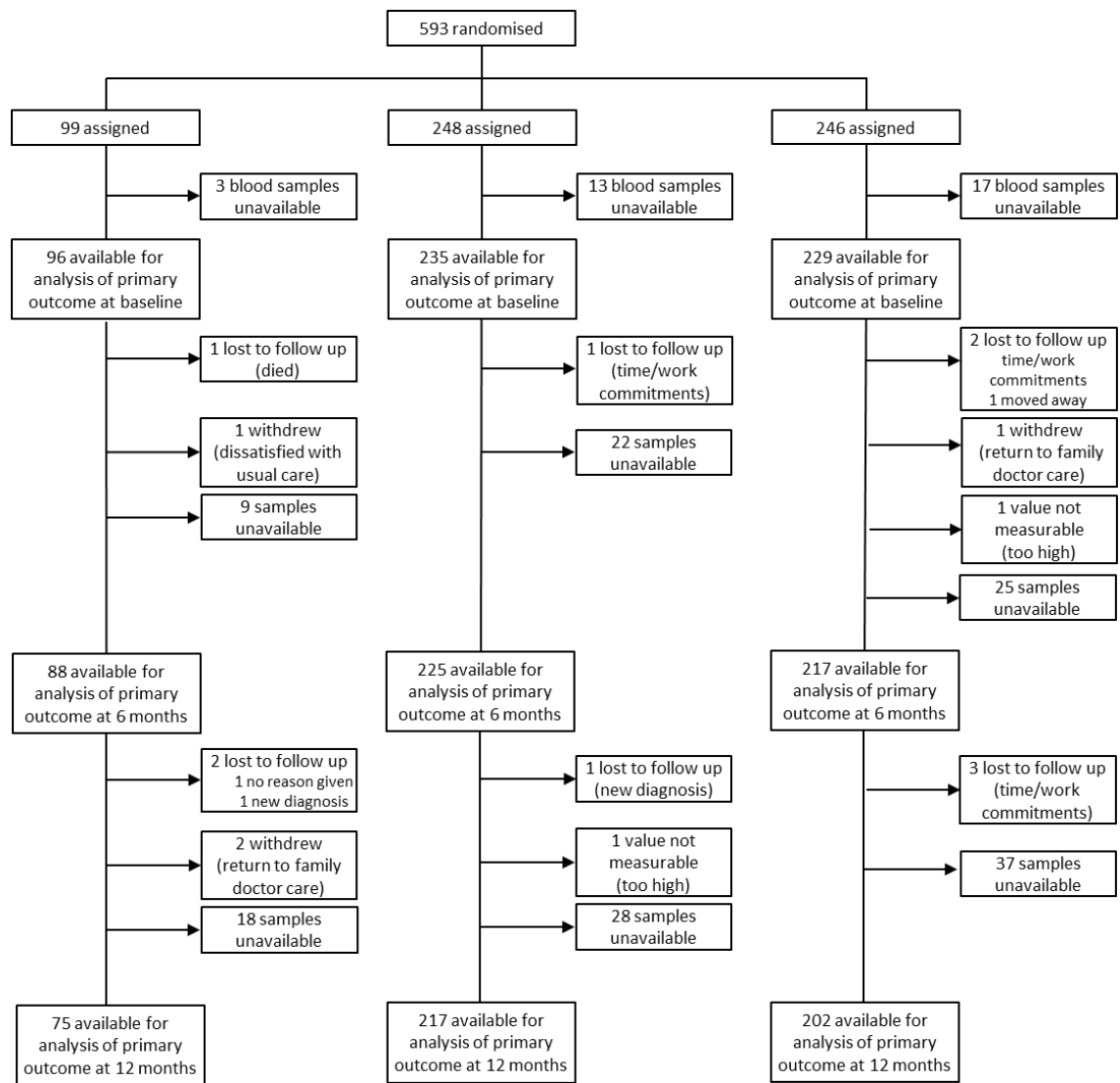


Figure 3.1: Trial profile

3.3.2 Inflammatory markers at 6 months

Adjusted CRP, IL-6 and sICAM-1 fell in both intervention arms in comparison to usual care. The reductions in CRP and sICAM-1 were statistically significant but not different between arms. Although the reduction in IL-6 in the diet and diet plus activity group just fell short of being statistically significant according to the 95 % confidence interval, both groups achieved a significant reduction similar to the reduction seen in other inflammatory markers. Adiponectin increased in both intervention arms relative to usual care, this increase was statistically significant but not different between arms. Overall, both interventions *versus* usual care produced similar effects on these markers, with a trivial difference between interventions as shown by Table 3.2. The percentage

change from baseline to 6 months *versus* usual care for each inflammatory marker is displayed in Figure 3.2.

Table 3.2: Adjusted means for all outcomes at the 6-month time-point. The geometric means are adjusted for the baseline value of the outcome, all minimisation variables (age, sex, fitness, route into the study, blood pressure), and study centre. The adjusted means are expressed with additional precision to better illustrate differences between geometric means expressed as a ratio.

Outcome	Diet	Diet plus Activity	Usual Care	Between-arm difference (95 % confidence interval)
CRP (mg/l)	1.73	1.70	2.18	
Diet plus activity vs. usual care				-22 (-38 to -3.1) %
Diet vs. usual care				-21 (-36 to -1.4) %
Diet plus activity vs. diet				-1.9 (-17 to 16) %
IL-6 (pg/ml)	2.06	2.11	2.29	
Diet plus activity vs. usual care				-7.8 (-19 to 4.4) %
Diet vs. usual care				-10 (-21 to 1.9) %
Diet plus activity vs. diet				2.5 (-6.7 to 13) %
sICAM-1 (ng/ml)	236.7	235.4	249.7	
Diet plus activity vs. usual care				-5.8 (-9.7 to -1.6) %
Diet vs. usual care				-5.2 (-9.2 to -1.1) %
Diet plus activity vs. diet				-0.6 (-3.7 to 2.7) %
Adiponectin (µg/ml)	5.53	5.45	5.12	
Diet plus activity vs. usual care				6.5 (0.8 to 13) %
Diet vs. usual care				8.2 (2.3 to 14) %
Diet plus activity vs. diet				-1.5 (-5.5 to 2.8) %

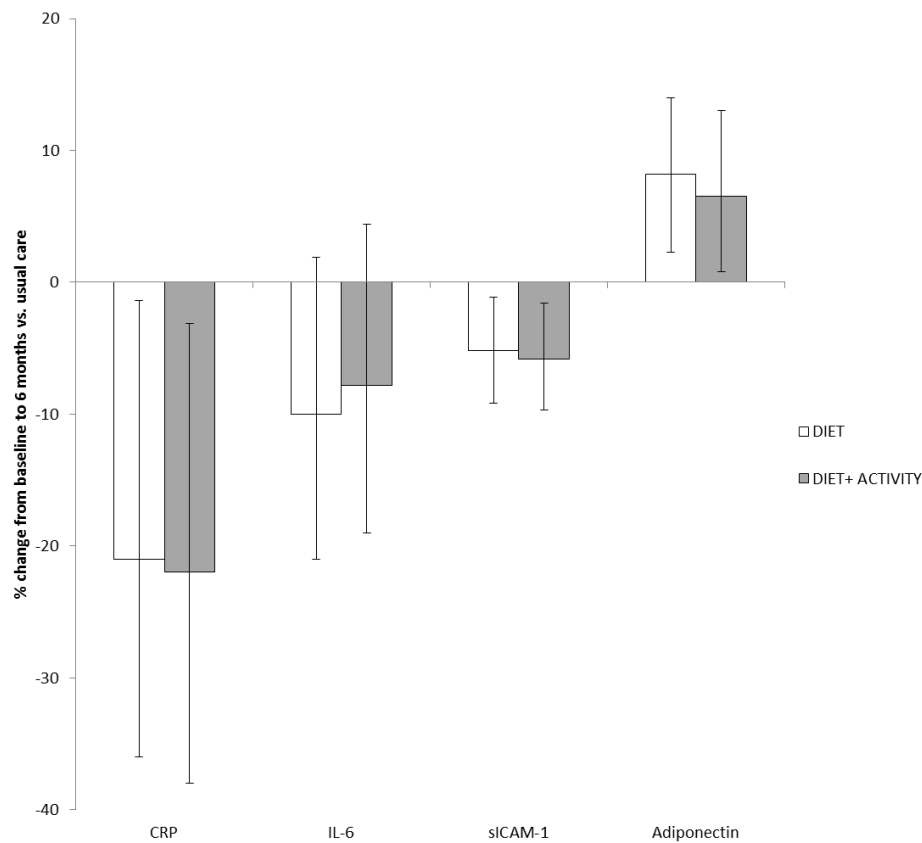


Figure 3.2: Percentage change from baseline to 6 months *versus* usual care for each inflammatory marker in the diet and diet plus activity arms. Data shown with 95 % confidence intervals.

3.3.3 Mediators

We have examined the mediating effect of body mass, HbA_{1c} and HOMA-IR and these are presented in a Table 3.3. The change in body mass had the biggest effect on adiponectin mediating ~60 % of the changes. It also had large impact on CRP mediating almost half of the total effect. The impact on sICAM-1 and IL-6 was less pronounced with the change in body mass only mediating ~30 % and ~20 % of the changes in those markers, respectively. Changes in HOMA-IR mediated ~30 % of the changes in all inflammatory markers except for IL-6 where it mediated <10 % of the changes.

Table 3.3: Mediation of total effects at 6 months by three putative mechanism variables. The percentage shown is the mean proportion of the total effect from Table 3.2 mediated by the mechanism variable.

Outcome	Δ Body Mass	Δ Hba_{1c}	Δ HOMA-IR
CRP			
Diet plus activity	43 %	31 %	30 %
Diet	47 %	25 %	32 %
IL-6			
Diet plus activity	22 %	7 %	8 %
Diet	17 %	4 %	6 %
sICAM-1			
Diet plus activity	26 %	24 %	28 %
Diet	31 %	21 %	31 %
Adiponectin			
Diet plus activity	68 %	25 %	42 %
Diet	56 %	15 %	34 %

3.3.4 Correlations

The strength of relationships between changes in parameters is displayed in Table 3.4. Changes in weight were positively correlated with the changes in several parameters such as blood pressure (systolic and diastolic), waist circumference, HbA_{1c}, TAG, glucose, insulin, HOMA-IR, sICAM-1, CRP and inversely correlated with changes in HDL cholesterol, adiponectin and activity. Changes in HbA_{1c} were positively correlated with the changes in weight, waist circumference, total cholesterol, TAG, glucose, HOMA-IR, sICAM-1, CRP and inversely correlated with changes in HOMA-β and adiponectin. Changes in glucose were positively correlated with the changes in weight, waist circumference, HbA_{1c}, TAG, insulin, HOMA-IR, HOMA-β, sICAM-1, CRP and inversely correlated with changes in HDL cholesterol and adiponectin. Changes in CRP were positively correlated with the changes in weight, waist circumference, HbA_{1c}, glucose and inversely correlated with changes in HOMA-β.

Table 3.4: Pearson rank correlation between changes in parameters. ** denotes a correlation at the 0.01 level (2-tailed) * denotes a correlation at the 0.05 level (2-tailed). Sys BP= Systolic blood pressure, Dia BP= Diastolic blood pressure, Waist cir= Waist circumference, HbA_{1c}= Glycated haemoglobin, Total chol= Total cholesterol, LDL chol= Low-density lipoprotein, HDL chol= High-density lipoprotein, TAG= Triacylglycerol, HOMA-IR= Homeostasis model assessment of insulin resistance, HOMA- β = Homeostasis model assessment of β -cell function.

	Sys BP	Dia BP	Weight	Waist cir	HbA _{1c}	Total chol	LDL chol	HDL chol	TAG	Glucose	Insulin	HOMA-IR	HOMA- β	Activity	sICAM-1	IL-6	CRP	Adiponectin
Sys BP																		
Dia BP	.704**																	
Weight	.116**	.115**																
Waist	.024	-.013	.574**															
HbA _{1c}	-.011	.081	.331**	.252**														
Total chol	.118**	.221**	.098*	.034	.139**													
LDL chol	.112*	.237**	.052	.021	.040	.888**												
HDL chol	.026	.027	-.148**	-.125*	-.096	.171**	-.123*											
TAG	.043	.032	.195**	.130**	.177**	.387**	.023	-.214**										
Glucose	.068	.098*	.318**	.202**	.690**	.222**	.111*	-.127**	.252**									
Insulin	-.010	.007	.282**	.213**	.047	.029	-.010	-.047	.176**	.196**								
HOMA-IR	.028	.055	.349**	.242**	.260**	.072	.006	-.092	.224**	.512**	.911**							
HOMA- β	-.037	-.079	.017	-.020	-.232**	-.045	-.070	.024	.110*	-.333**	.633**	.359**						
Activity	.064	-.034	-.125**	-.078	-.069	.014	.029	.040	-.007	-.046	-.048	-.064	.003					
sICAM-1	-.006	.025	.184**	.109*	.224**	-.016	.006	-.159**	.060	.184**	.085*	.162**	-.092*	-.019				
IL-6	.000	.011	-.006	-.027	.051	-.024	-.006	-.081	-.008	.088*	-.019	.023	-.045	.016	.165**			
CRP	.053	.024	.127**	.135**	.169**	-.108*	-.105*	.040	-.085	.135**	-.066	.020	-.216**	.034	.249**	.355**		
Adiponectin	-.001	-.023	-.300**	-.151**	-.156**	.151**	.101*	.258**	-.005	-.139**	-.105*	-.149**	.046	.001	.014	-.004	-.072	

3.3.5 Changes in inflammatory markers at 12 months

The effects for CRP and adiponectin were weakened at 12 months and were no longer statistically different to usual care; although this was mainly due to improvements in the usual care group. Effects for sICAM-1 remained. IL-6 concentrations decreased further in the diet arm and were statistically significant as shown in Table 3.4.

Table 3.4: Adjusted means for all outcomes at the 12-month time-point. The geometric means are adjusted for the baseline value of the outcome, all minimisation variables (age, sex, fitness, route into the study, blood pressure), and study centre. The adjusted means are expressed with additional precision to better illustrate differences between geometric means expressed as a ratio.

Outcome	Diet	Diet plus Activity	Usual Care	Between-arm difference (95 % confidence interval)
CRP (mg/l)	1.57	1.60	1.73	
Diet plus activity vs. usual care				-7.6 (-26 to 16) %
Diet vs. usual care				-9.6 (-28 to 13) %
Diet plus activity vs. diet				2.2 (-13 to 20) %
IL-6 (pg/ml)	1.95	2.03	2.24	
Diet plus activity vs. usual care				-9.3 (-21 to 3.8) %
Diet vs. usual care				-13 (-24 to -0.8) %
Diet plus activity vs. diet				4.5 (-5.3 to 15) %
sICAM-1 (ng/ml)	232.3	235.0	250.5	
Diet plus activity vs. usual care				-6.2 (-10 to -2.1) %
Diet vs. usual care				-7.3(-11 to -3.3) %
Diet plus activity vs. diet				1.2 (-2.0 to 4.4) %
Adiponectin (µg/ml)	5.59	5.56	5.31	
Diet plus activity vs. usual care				4.6 (-3.5 to 13) %
Diet vs. usual care				5.2 (-2.8 to 14) %
Diet plus activity vs. diet				-0.6 (-6.3 to 5.4) %

3.4 Discussion

These results from Early ACTID show that motivational unsupervised diet and diet plus physical activity interventions in patients with newly-diagnosed type 2 diabetes lead to reductions in CRP, IL-6 and sICAM-1 with an increase in adiponectin. There was no greater benefit from adding physical activity advice to dietary advice. Approximately half of the observed effect for the primary outcome (CRP) was explained by the change in weight. It is noteworthy that a relatively modest 2-3 % decrease in weight led to a fall in circulating CRP of approximately 20 %. These results are the first to show that simple diet and diet/physical activity interventions can be integrated into real-world health care settings and generate beneficial changes in various inflammatory markers in early type 2 diabetes.

Both intervention arms experienced changes in CRP, IL-6, sICAM-1 and adiponectin that were greater than usual care after 6 months. However, over the following 6 months, the changes in CRP and adiponectin in the usual care arm were relatively greater than the intervention arms so that there were no longer any differences when compared to usual care at 12 months. This was not the case for sICAM-1, which remained different in both intervention arms when compared to usual care at 12 months. Importantly, during the first 6 months of Early ACTID, there were no changes to medication whereas between 6 and 12 months medication was adjusted to target pre-defined clinical outcomes. As discussed previously, there was a greater increase in diabetes medication use in the usual care arm of Early ACTID than either intervention arm (Andrews *et al.*, 2011). Diabetes medication such as metformin has an established effect on inflammatory markers such as CRP (Dandona *et al.*, 2004). Thus, for CRP and adiponectin, conventional treatment appears to catch up with diet and diet plus physical activity advice when medication is adjusted according to fixed protocols (Andrews *et al.*, 2011). However, conventional treatment was not as effective as diet and diet plus physical activity advice at improving sICAM-1 after 12 months.

The lack of additional effect from physical activity was surprising but similar to that reported for other outcomes in Early ACTID such as HbA_{1c} (Andrews *et*

al., 2011). Other studies show that *supervised* exercise can have a much greater effect on inflammatory markers than those reported in the present study (Xydakis *et al.*, 2004; Balducci *et al.*, 2010; Jorge *et al.*, 2011; Kadoglou *et al.*, 2013). However, Early ACTID is a pragmatic trial designed to test the benefit from giving advice on physical activity in addition to diet in a real-world, community-based setting in the first year after diagnosis. Thus, these results show the effect from this additional advice and not the effect of physical activity *per se*. This advice led to a significant but modest increase in physical activity (Andrews *et al.*, 2011). As a result of the energetics of physical activity and other factors such as substitution, a reasonably large change in a given behaviour might not translate into a large change in energy expenditure (Thompson *et al.*, 2012). Patients in the diet plus physical activity arm increased their step count by approximately 1300 steps per day (Andrews *et al.*, 2011). An increase in physical activity of 1300 steps·d⁻¹ would equate to walking approximately 1000 m with an energy cost of ~50 kcal per day (Thompson *et al.*, 2006). Thus, our results show that this change in physical activity has no additional benefit than dietary advice on the various inflammatory markers included in the present study. Investing in strategies to increase physical activity further may have yielded greater improvements but of course with a greater financial cost.

Since weight loss in these patients was responsible for much of the change in CRP and adiponectin in both intervention arms then, arguably, the primary focus for these outcomes should be the achievement of weight loss through any means. In support of this contention, one small study found that plasma adipokines are disturbed in obese but not non-obese patients with type 2 diabetes (Hansen *et al.*, 2010). Furthermore, studies have shown that adiposity and not differences in physical activity explain variation in circulating markers of inflammation (Dixon *et al.*, 2013). Our data supports this as changes in weight were correlated with changes in adiponectin, sICAM-1 and CRP while changes in activity were not. Exercise without pronounced weight loss does not change concentrations of CRP (Church *et al.*, 2010; Stewart *et al.*, 2010; Thompson *et al.*, 2010; Fisher *et al.*, 2011). Thus, although weight loss was relatively modest in diet and diet plus physical activity arms during the first 6

months, this was sufficient to produce beneficial changes in CRP and adiponectin. The lack of any further weight loss over the following 6 months may explain why there was no further improvement at 12 months in either intervention arm for CRP and adiponectin.

In contrast to the findings for CRP and adiponectin, the change in sICAM-1 was sustained over 12 months in diet and diet plus physical activity arms. This is similar to the sustained changes in HbA_{1c}, fasting insulin and fasting glucose reported previously (Andrews *et al.*, 2011). It is also noteworthy that the change in weight was a less important mediator of the change in sICAM-1 than for CRP and adiponectin. Measurement of sICAM-1 independently predicts the risk of cardiovascular disease and stroke in people with Type 2 Diabetes (Shai *et al.*, 2006; Kanai *et al.*, 2008). Thus, diet and diet plus physical activity is more effective than conventional treatment at targeting inflammatory markers associated with vascular risk in patients with type 2 diabetes (Shai *et al.*, 2006; Kanai *et al.*, 2008).

Two of the strengths of Early ACTID are (i) it purposefully targeted patients with newly-diagnosed type 2 diabetes and (ii) there was no change in medication over the first six months in order to better elucidate the impact of lifestyle intervention. It is noteworthy that average CRP was in the 'moderate' risk category according to established thresholds for CRP (Pearson *et al.*, 2003; Ridker *et al.* 2003) and naturally the findings may have been different in patients with more established diabetes and/or higher baseline values. We should also highlight that whilst the changes in inflammatory measures were statistically significant, the size of the effects were small to modest. However, it is also important to highlight that this was accomplished with a modest change to lifestyle achieved without supervision and in a community-based setting.

The findings from this pragmatic research will help improve clinical practice and future patient care. We have shown that a modest reduction in weight resulting from diet alone or diet combined with increase physical activity has led to tangible positive changes in markers of inflammation. The increase in moderate to vigorous physical activity achieved between baseline and 6 months

in the diet plus activity group was modest (10 min per day compared to 3 min per day for the diet arm). This was likely to be too small to separate the relative importance of physical activity compared to changes in energy balance and their separate impact on metabolic and inflammatory health outcomes.

Our results indicate that motivational, unsupervised, community-based diet and diet plus physical activity interventions can be integrated into real-world health care settings and generate beneficial changes in various markers of inflammation in patients with newly-diagnosed type 2 diabetes. Once adjustments to medication were permitted (i.e., after 6 months), usual care was equally effective at improving CRP and adiponectin but not sICAM-1. Thus, diet and diet plus physical activity advice were more effective at improving markers of vascular risk than conventional drug therapy. Alternative models carefully designed to investigate the effect of exercise on inflammatory markers and metabolic control independent of changes in energy balance may be useful to clarify the benefits of physical activity *per se*.

CHAPTER 4

Exercise counteracts short-term overfeeding and reduced physical activity independent of any net attenuation of energy imbalance in healthy young men.

4.1 Introduction

Western lifestyles are typified by chronically low levels of physical activity and excessive caloric intake, resulting in positive energy balance (Hill & Wyatt, 2005; Uauy & Diaz, 2005) and an accumulation of triacylglycerol within adipocytes. Adipose tissue is not just a site of energy storage and acts as an endocrine organ capable of secreting growth factors and adipokines (Rajala & Scherer, 2003) with implications for metabolic control via adipocyte insulin resistance and subsequent elevations in blood lipids (Khan *et al.*, 2006). This provides one mechanism for the established link between energy imbalance, obesity and insulin resistance (Horowitz, 2007).

Intermittent and/or sustained periods of overconsumption and/or under-activity are necessarily responsible for net positive energy balance. Numerous studies have examined overfeeding of varied duration and composition, with the resultant positive energy balance associated with impaired insulin sensitivity (Wang *et al.*, 2001; Cornier *et al.*, 2006; Brons *et al.*, 2009) and marked alterations in adipose tissue gene expression (Shea *et al.*, 2009; Alligier *et al.*, 2012). This in turn impacts the systemic concentration of key adipokines involved in the regulation of energy balance, such as leptin (Lammert *et al.*, 2000; Wang *et al.*, 2001; Brons *et al.*, 2009), adiponectin (Brons *et al.*, 2009) and visfatin (Sun *et al.*, 2007). Equally, enforced physical inactivity results in impaired insulin sensitivity and other negative health outcomes in previously healthy, active individuals (Vukovich *et al.*, 1996; Arciero *et al.*, 1998; Olsen *et al.*, 2008).

In contrast to the relative wealth of information in relation to the separate influences of overfeeding or physical inactivity, it is remarkable that only two studies have explored the combined impact of both factors applied

simultaneously. A recent study by Knudsen *et al.* (2012) demonstrated impaired insulin sensitivity after just 3 days of overfeeding (150 % habitual intake) combined with restricted step count (≤ 1500 steps per day), an effect that preceded any measurable change in body composition. Previously, Hagobian & Braun (2006) reported similarly rapid metabolic dysregulation in a cohort of habitually-active participants provided with 125 % of their habitual intake for 3 days whilst abstaining from structured exercise. Importantly, however, insulin responses were restored to baseline levels by a single bout of moderate-intensity exercise, despite additional overfeeding on that day to match for the energy expended during exercise. This finding presents the interesting possibility that prescribed daily exercise may offset the negative health effects of overfeeding plus reduced physical activity independent of any impact on energy (im)balance.

Thus, we hypothesised that short-term combined overfeeding and reduced physical activity would significantly impair metabolic function but that the incorporation of daily physical exercise within the same experimental model would prevent these changes (independent of any net effect on energy balance). Furthermore, given the potential independent effects of energy balance and exercise on adipose tissue function given its role in energy storage (Thompson *et al.*, 2012); we hypothesised that changes in the expression of key genes within adipose tissue in response to a standardised energy surplus would be offset by daily vigorous-intensity exercise.

4.2 Methods

4.2.1 Approach to the Research Question

The purpose of this study was to examine whether daily vigorous-intensity exercise would offset the metabolic consequences of short-term overfeeding and reduced physical activity, independent of any net attenuation of energy imbalance. A randomised parallel group design was used for this study (registration number: ISRCTN59822195), with participants randomly allocated by a third party to experience a fixed energy surplus via seven days of overfeeding and restricted physical activity *per se* (SUR) or with a daily bout of physical exercise (SUR+EX). The SUR group reduced their daily activity to

≤ 4000 steps while increasing their habitual energy intake by 50 % (Chapter 2.9). The SUR+EX group adhered to a matched model of overfeeding and reduced physical activity except for the introduction of a daily bout of vigorous-intensity treadmill running (5 min warm-up at 60 % $\dot{V}O_{2\max}$ then 45 min at 70 % $\dot{V}O_{2\max}$), with an additional overfeed individually prescribed to account for the energy expended during each run and thus standardise the energy surplus (i.e. habitual energy intake increased by 75 ± 3 %). The daily step allowance (4000 steps) was selected to allow some margin of error without exceeding the 5000 steps per day thought to reflect a sedentary lifestyle (Tudor-Locke *et al.*, 2000).

The extent of overfeeding selected was based on the balance of available literature regarding overfeeding in general, along with our preliminary work that supported this as a realistic and thus practically achievable target for both groups. While previous studies have examined overfeeding of varied nature (e.g. mixed, high carbohydrate, high fat, *etc.*), we elected to overfeed each individual according to their habitual diet and thus avoid introducing an additional variable at this stage. Changes in insulin sensitivity and glycaemic control (reflected by basal values and incremental Area Under the Curve (iAUC) measured at baseline and follow up were the primary outcome measures. Other blood measures related to metabolic function, inflammation and changes within adipose tissue were secondary outcome measures.

4.2.2 Participants

Twenty-six healthy, habitually active men completed the study. Participants were 25 ± 7 years old (mean \pm SD) and had been weight stable (± 3 kg) for at least 6 months. Only volunteers who undertook structured vigorous-intensity exercise for thirty minutes or more, at least three times a week were included in the study, assessed via a self-report questionnaire. Participants completed a health questionnaire (Appendix 1) and Physical Activity Readiness Questionnaire (PAR-Q; Appendix 3) to determine eligibility and provided written and verbal consent consistent with the requirements of the Bath NHS Research Ethics Committee, who approved this study (REC reference number : 07/H0101/234). Individuals who smoked, suffered from a condition known to interact with the study measures or took regular medication that may have

interfered with the results were excluded from the study. The anthropometric and physiological characteristics of those included are presented in Table 4.1.

Table 4.1: Participant details. Mean \pm SD

Characteristic	SUR group (n=14)	SUR + EX group (n=12)
Age (y)	25 \pm 7	26 \pm 7
Height (m)	1.80 \pm 0.07	1.79 \pm 0.05
Mass (kg)	75.5 \pm 8.9	78.2 \pm 11.0
BMI (kg·m ⁻²)	23.2 \pm 2.5	24.4 \pm 2.6
FMI (kg·m ⁻²)	3.3 \pm 1.2	3.8 \pm 1.1
$\dot{V}O_{2\max}$ (ml·kg ⁻¹ ·min ⁻¹)	58.7 \pm 5.9	54.9 \pm 4.8
$\dot{V}O_{2\max}$ (l·min ⁻¹)	4.4 \pm 0.8	4.3 \pm 0.5

4.2.3 Design Summary

Participants were randomly allocated by a third party to one of two groups for the study design of seven days using a block randomisation plan with age stratification (18-28 or 29-40 years):

- 1) Overfeeding with restricted physical activity (SUR) n=14
- 2) Overfeeding with restricted physical activity alongside a daily exercise bout (SUR+EX) n=12

The planned experimental design is summarised below (Figure 4.1).

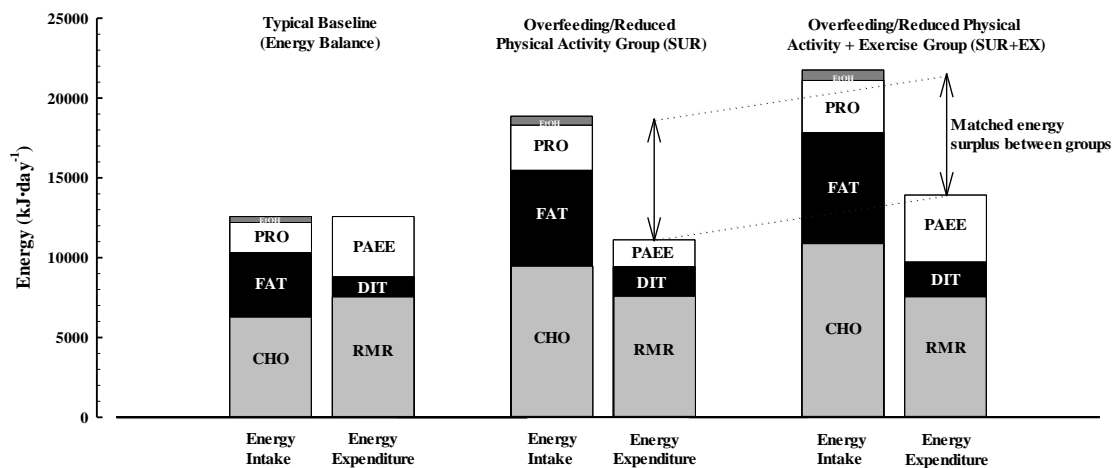


Figure 4.1: Schematic representation of the planned energy surplus that will be induced by the overfeeding and restricted physical activity model. CHO= Carbohydrates, PRO= Protein, EtOH= Alcohol, RMR= Resting Metabolic Rate, DIT= Diet Induced Thermogenesis, PAEE= Physical Activity Energy Expenditure.

All participants performed a 30 min bout of running the day before they were due to start the trial, either in the morning or at lunch time but no later than 2pm in order to control for an acute effect of exercise. Participants recorded their step count using a pedometer (Yamax, Japan) for three days leading up to and during the whole trial. All participants reduced their steps to ≤ 4000 steps·day⁻¹ for the duration of the trial. During the intervention, PAEE was estimated using individual average step count as combined accelerometry and heart rate (Actiheart, CamNtech, Cambridge UK) data proved to be confounded. It appears that overfeeding confounds the assumptions underlying the estimation of energy expenditure via this method. The average sleeping heart rate (mean \pm SD) went from 47 ± 7 beats·min⁻¹ to 49 ± 8 beats·min⁻¹ in the SUR group (n=12) and from 53 ± 4 beats·min⁻¹ to 56 ± 5 beats·min⁻¹ in the SUR+EX group (n=11). This finding has already been reported after long-term overfeeding in identical twins by Bouchard *et al.* (1990). Whilst this effect might appear modest, this has a profound impact on estimated PAEE using this methodology. As a result, data from the Actiheart was solely used to ensure compliance (i.e., presence or absence of structured exercise). The volunteers in the SUR+EX group were required to perform 45 min of treadmill running each day at 70 % of their maximum oxygen uptake preceded by a 5 min warm-up. Importantly, this group received additional prescribed energy intake (Chapter 2.9) to account for the energy expended during the exercise. The Excess Post-Exercise Oxygen Consumption (EPOC) associated with each exercise bout was taken into account when prescribing the overfeeding to the SUR+EX group and was estimated as 6.6 % of energy expended during the run based on LaForgia *et al.* (2006). The DIT associated with the extra food prescribed to the SUR+EX group compared to the SUR group was also taken into consideration and was calculated as 10 % of the energy expended during the run (including EPOC) based on Westerterp (2004). Finally, the contribution of RMR towards total energy expenditure during each run (50 min) was subtracted from the overfeeding calculation as this was already taken into account when prescribing the 50 % overfeed. An example of an overfeeding calculation for an individual from each group in energy balance (12570 kJ/3000 kcal) at baseline is shown below (Table 4.2).

Table 4.2: An example of an overfeeding calculation is shown below. EPOC = 6.6 % of energy expended during run based on LaForgia *et al.* (2006). DIT = 10 % of extra energy intake supplied + EPOC based on Westerterp (2004). RMR for the duration of the run.

	SUR group	SUR+EX group
Average daily energy intake	12570 kJ	12570 kJ
50 % overfeeding	+ 6285 kJ	+ 6285 kJ
Energy expended during run	n/a	+ 2933 kJ
EPOC	n/a	+ 194 kJ
DIT (additional overfeeding)	n/a	+ 313 kJ
RMR (during run)	n/a	- 293 kJ
Total energy intake prescribed	18855 kJ	22002 kJ
Surplus	6285 kJ	6285 kJ

Participants abstained from tea/coffee or alcohol the day before each trial. During the days leading up to the trial, volunteers exercised as normal and consumed their habitual diet. The final exercise bout prescribed under the SUR+EX treatment was performed at a standardised time of day specific for each participant. This took place at the same time as the running bout each individual had performed the day before baseline measures were taken. All participants performed this bout of exercise either in the morning or at lunchtime but not later in the day.

4.2.4 Laboratory Measurements

Following an overnight fast (≥ 10 h), participants arrived in the laboratory at 0700 ± 0.5 h. Body mass was measured to the nearest 0.1 kg using electronic scales (Tanita Corporation, Japan). All participants wore the same pair of shorts at baseline and follow-up on top of their underwear for body mass measurement. Anthropometric measurements were made in triplicate using a metallic tape measure (Lufkin, US) before lean and fat mass were measured using Dual-Energy X-ray absorptiometry (DEXA) (Discovery, Hologic, Bedford, UK) (Chapter 2.2). RMR was estimated by indirect calorimetry from expired air samples. Four 5-min samples were collected in Douglas bags, with additional samples collected if values were not stable within $100 \text{ kcal} \cdot \text{day}^{-1}$ (Compher *et al.*, 2006; Betts *et al.*, 2011). Once four stable values had been

collected, the lowest was accepted as RMR. Following the application of a topical local anaesthetic (1.5 ml Ametop gel, Smith & Nephew, Hull, England) an 18-gauge 1.3 x 45 mm cannula (BD Venflon Pro) was inserted into an antecubital vein and a baseline blood sample collected. An adipose tissue sample was then collected (Chapter 2.10) before an OGTT was performed according to the procedure described in Chapter 2.4. Blood pressure was measured in triplicate at the end of each trial (Chapter 2.3). The same protocol was used at follow-up.

4.2.5 Calculation of energy surplus (post-intervention)

The energy surplus induced by the overfeeding and restricted physical activity model was quantified retrospectively post-intervention based on the following assumptions. For each macronutrient, we assumed that the amount entering the system was equal to the amount ingested (i.e. ignoring urinary and faecal losses). The factors 16.76, 29.33, 37.29 and 15.71 kJ·g⁻¹ (4, 7, 8.9 and 3.75 kcal·g⁻¹) were used to calculate the metabolised energy contents of protein, alcohol, fat and carbohydrate, respectively (Bender, 2006). We estimated DIT to be 21 % for protein, 15 % for alcohol, 2 % for fat and 8 % for carbohydrate based on Westerterp (2004). Based on these values, DIT was calculated retrospectively post-intervention to be 8.3 ± 0.6 % of energy intake for the population who took part in this study. Resting carbohydrate and fat oxidation were based on indirect calorimetry measurements collected in a fasted state at baseline and follow-up. Where Respiratory Exchange Ratio (RER) was >1, the apparently negative rates of lipid oxidation were assumed to reflect quantitatively proportional lipid synthesis (Frayn, 1983). The amount of lipid synthesised was added to the fat intake of each participant for calculations of substrate balance. It was assumed that 1 g of glucose was required to produce 0.52 g of lipid based on Ferrannini (1988). Any minor PAEE during the intervention was calculated as the product of average daily step count and an assumed stride length of 0.825 m (Auvinet *et al.*, 2002) based on an oxygen cost of 0.125 ml·m⁻¹·kg⁻¹ (Dill, 1965). Exclusive reliance on carbohydrates as a fuel source during physical activity was assumed as participants mean RER during overfeeding was 0.98 even under fasted and resting conditions at follow-up. The energy cost associated with the exercise sessions was based on the indirect

calorimetry measurements during participants' preliminary exercise tests taking part in the study. Again, exclusive reliance on carbohydrates as fuel source during running was assumed. The overall energy surplus was estimated by calculating the sum of the surpluses associated with each macronutrient. The calculated energy surplus for all participants is provided in Appendix 4.

4.2.6 Analytical methods

Blood was analysed for WBC counts, serum CRP, IL-6, Adiponectin, Leptin, ALT, Insulin, C-peptide, TAG, Cholesterol, HDL-Cholesterol, NEFA and plasma glucose. Adipose tissue was analysed for mRNA (Chapter 2.11, 2.12) and protein expression profiles.

4.2.7 Western Blot Analysis

One ml of organic phase was removed and mixed with 1.5 ml of Isopropanol before being inverted several times and incubated for 10 min at room temperature. Samples were spun for 10 min at 2500 g, 4°C. Supernatants were discarded and pellets were washed using a 0.3 M Guanadine HCl in a 95 % Ethanol solution and incubated with rotation for 20 min at room temperature. Samples were then spun for 10 min at 2500 g, 4°C and supernatant removed. This wash procedure was repeated 3 times. After the final wash, pellets were mixed with 2 ml of 100 % Ethanol and left for 20 min at room temperature before being spun for 10 min at 2500 g, 4°C. Supernatants were removed and the pellet was left to air dry. 400 µl of a 1 % SDS solution was added to the pellets before sonication (18 µm) on ice. Protein content was determined by BCA protein assay (Thermo Scientific). 20 µg of the protein solution was loaded on a SDS-Polyacrylamide gel for electrophoresis alongside Novex® sharp protein standard (Life Technologies, UK). This was carried out using a modification of the method of Hashimoto *et al.* (1983), using the discontinuous buffer system of Laemmli (Laemmli *et al.*, 1970). 6 % acrylamide Tris-Glycine SDS-PAGE slab gels were prepared using the triple-wide mini-vertical electrophoresis systems (CBS Scientific, USA). Gels were run at 200 V for about 1 h until the bromophenyl blue tracking dye had just run off the gel. Proteins were then transferred to a nitrocellulose membrane. The nitrocellulose membrane was washed in Tris-buffered saline containing Tween-20 (TBS-T) to remove the

Ponceau stain. The membrane was blocked using a 5 % (w/v) Marvel (dried skimmed milk powder) in TBS-T for 60 min to block non-specific protein sites before being washed four times in TBS-T for 15 min at a time. It was then incubated with the primary antibody overnight at 4°C. The antibody was diluted in 1 % BSA in TBS-T: Akt1 (Millipore), Akt2 (Cell Signaling Technology), GLUT4 (Holman *et al.*, 1990), AMPK (Cell Signaling Technology), GAPDH (Millipore) or 5 % BSA TBS-T: pAKT(Cell Signaling Technology), pAMPK (Cell Signaling Technology). The nitrocellulose membrane was then washed six times with TBS-T for 5 min at a time and then incubated with a secondary antibody for 1 h at room temperature. The secondary antibody was diluted in 5 % Marvel-TBS-T. Goat anti-rabbit IgG HRP conjugate (Millipore, USA) or Goat anti-mouse IgG HRP conjugate (Thermo scientific, USA) were used at a 1:4000 dilution. Finally, the nitrocellulose was washed six times in TBS-T for 5 min at a time and developed using ECL or ECL Advance (GE healthcare) following the manufacturer's guidelines. Solutions were mixed in a 1:1 ratio and the membrane incubated for 1 min for ECL and 5 min for ECL advance. The image was detected by use of a Hamamatsu camera attached to the EpiChemi II Darkroom (UVP).

4.2.8 Statistical analysis

To simplify data analysis and facilitate a more meaningful interpretation of an otherwise complex factorial research design (Hopkins *et al.*, 2009 & Matthews *et al.*, 1990), serial measurements of glucose, insulin and C-peptide at baseline and follow-up were converted into simple summary statistics to illustrate the net response of each parameter (i.e. within-subject peak concentrations, time to peak and incremental area under curve; Wolever & Jenkins, 1986). Pre-planned contrasts were conducted in relation to the absolute group differences both at final follow-up and the relative change from baseline. The precise time-course of responses within and between trials were analysed using factorial 2- and 3-way mixed-model analysis of variance (groupxday & groupxdayxtime, respectively) irrespective of minor deviations from a normal distribution (Maxwell & Delaney, 1990) but with the Greenhouse-Geisser correction applied to intra-individual contrasts for $\epsilon < 0.75$ and the Huynh-Feldt correction adopted for less severe asphericity (Atkinson, 2002). Where significant interactions were

observed, multiple t-tests were applied to determine the location of variance both between treatments at each time point and between time points within each treatment relative to baseline, with both methods subject to a Holm-Bonferroni correction (Atkinson, 2002). For all the above statistical approaches, statistical significance was set at an alpha level of $P \leq 0.05$. Data are presented in text as means and standard deviations (SDs). The table displays 95 % confidence intervals (95 % CI) to better illustrate group differences, whereas the variance bars on figures are normalised confidence intervals (nCI) that have been corrected to remove inter-individual variation (Masson, 2003). For reference, the magnitude of these CIs illustrate the change at each time point relative to baseline such that, in general, plotted means which CIs do not overlap by more than one-half of one side of an interval are likely to be deemed statistically different according to conventional significance testing (Masson, 2003). A main effect of day denotes an effect of energy surplus (†: Day 1 vs. Day 8 both groups), whereas a day \times group interaction means there is a mediating effect of exercise (*). Statistical analysis for the gene expression data was carried out on the ΔC_t values, whereas fold changes were used for the Western Blot data. The Pearson rank correlation was employed to determine the strength of relationships between parameters.

4.3 Results

Metabolic outcomes

4.3.1 Induced energy surplus

The estimated energy surplus induced by the overfeeding and restricted physical activity model is summarised in Figure 4.2. These were not statistically different ($P=0.16$) between groups ($11176 \pm 3699 \text{ kJ}\cdot\text{day}^{-1}$ and $9483 \pm 1815 \text{ kJ}\cdot\text{day}^{-1}$ in the SUR and SUR + EX group, respectively). At baseline, the average energy intake was $13485 \pm 2367 \text{ kJ}\cdot\text{day}^{-1}$ while the average energy expenditure was $14792 \pm 2849 \text{ kJ}\cdot\text{day}^{-1}$.

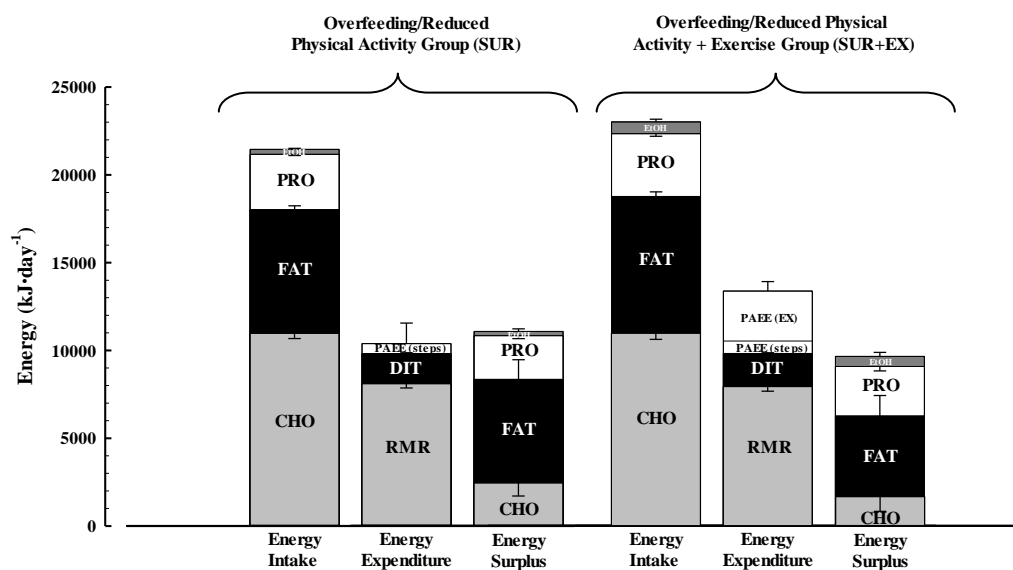


Figure 4.2: Schematic representation of the achieved energy surplus induced by the overfeeding and restricted physical activity model in SUR group ($n=13$) and SUR+EX group ($n=12$). Energy intake estimated from diet analysis. RMR estimated from indirect calorimetry. DIT was quantified post-intervention as 8.3 % of energy intake, this was done by calculating the specific DIT associated with each macronutrient before the overall DIT was determined. PAEE was based on step count and indirect calorimetry (exercise). Energy surplus was calculated as the difference between EI and EE. Values are means \pm nCI.

4.3.2 Diet composition

Diet composition before (Table 4.3) and during the intervention (Table 4.4) is summarised below. All participants confirmed that all prescribed foods had been consumed over the course of the intervention. These were not statistically different between groups.

Table 4.3: Habitual diet composition. Mean \pm SD

Characteristic	SUR group (n=13)	SUR + EX group (n=12)
Caloric intake (kJ·day ⁻¹)	13966 \pm 2885	12964 \pm 1604
Protein intake (kJ·day ⁻¹)	2103 \pm 431	2051 \pm 552
Carbohydrate intake (kJ·day ⁻¹)	7335 \pm 1789	6237 \pm 1217
Fat intake (kJ·day ⁻¹)	4331 \pm 1258	4308 \pm 698
Alcohol intake (kJ·day ⁻¹)	191 \pm 267	359 \pm 286

Table 4.4: Diet composition during the intervention. Mean \pm SD

Characteristic	SUR group (n=13)	SUR + EX group (n=12)
Overfeeding (%)	50	75 \pm 3
Caloric intake (kJ·day ⁻¹)	20949 \pm 4327	22697 \pm 2654
Protein intake (kJ·day ⁻¹)	3155 \pm 646	3596 \pm 990
Carbohydrate intake (kJ·day ⁻¹)	11002 \pm 2683	10917 \pm 2065
Fat intake (kJ·day ⁻¹)	6497 \pm 1887	7536 \pm 1142
Alcohol intake (kJ·day ⁻¹)	287 \pm 401	630 \pm 507

4.3.3 Physical activity levels

As discussed in the methods, overfeeding has an impact on heart rate. As a result, the assumptions underlying the estimation of energy expenditure using the Actiheart technology were confounded and step counts were used to quantify physical activity levels instead. Average steps significantly decreased during the intervention within the SUR and SUR+EX groups from 12562 ± 3520 to 3672 ± 860 steps·day⁻¹ and from 10544 ± 2756 to 3690 ± 400 steps·day⁻¹, respectively. There was a main effect of day ($F=120.0$, $P<0.001$) as shown by Figure 4.3.

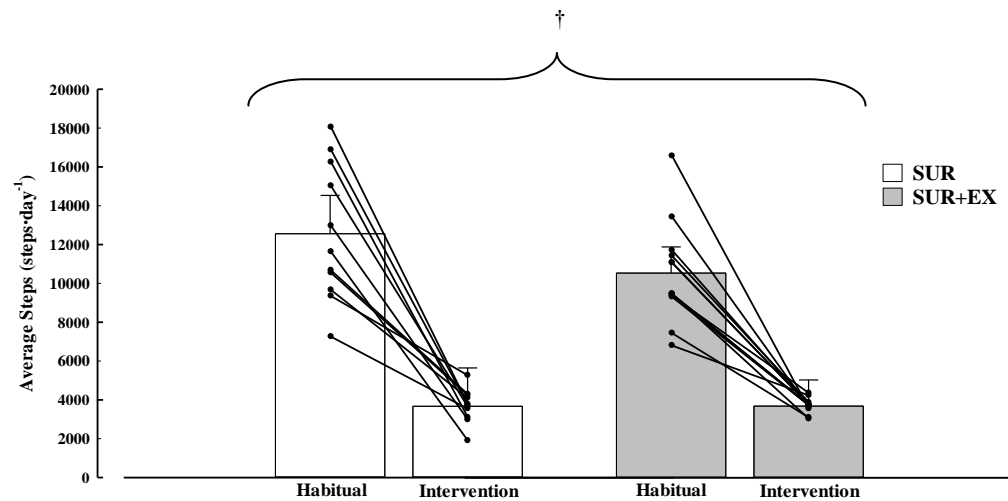


Figure 4.3: Schematic representation of the reduction in steps achieved during the intervention in SUR group (n=11) and SUR+EX group (n=11). Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=120.0$, $P<0.001$).

4.3.4 RMR & RER

Resting Metabolic Rate (RMR) significantly increased at follow-up in the SUR group from 7777 ± 1282 to 8619 ± 1161 kJ·day⁻¹ while it remained unchanged in the SUR+EX group from 8087 ± 1127 to 8275 ± 1131 kJ·day⁻¹. There was a main effect of day ($F=20.4$, $P<0.001$) and a day×group interaction ($F=8.2$, $P=0.009$) as shown by Figure 4.4. The RMR values described here were also used in Figure 4.2. Respiratory Exchange Ratio (RER; Figure 4.5) significantly increased at follow-up in the SUR and SUR+EX group, there was a main effect of day ($F=54.5$, $P<0.001$).

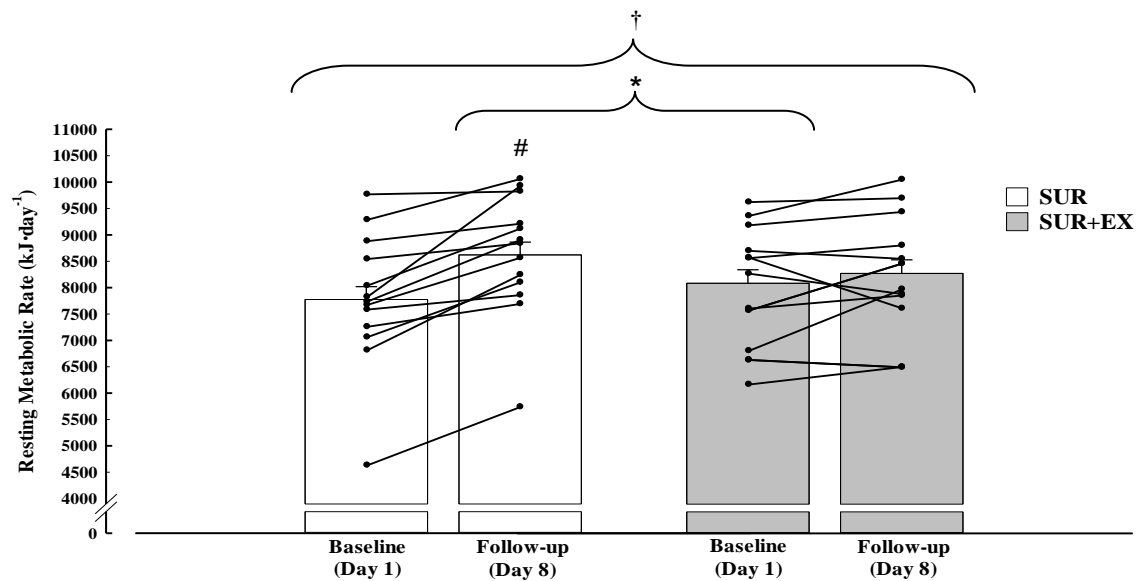


Figure 4.4: Resting metabolic rate at baseline and follow-up for the SUR group (n=13) and the SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=20.4$, $P<0.001$) * denotes a dayxgroup interaction ($F=8.2$, $P=0.009$) # denotes values different pre-post within SUR group ($P<0.001$).

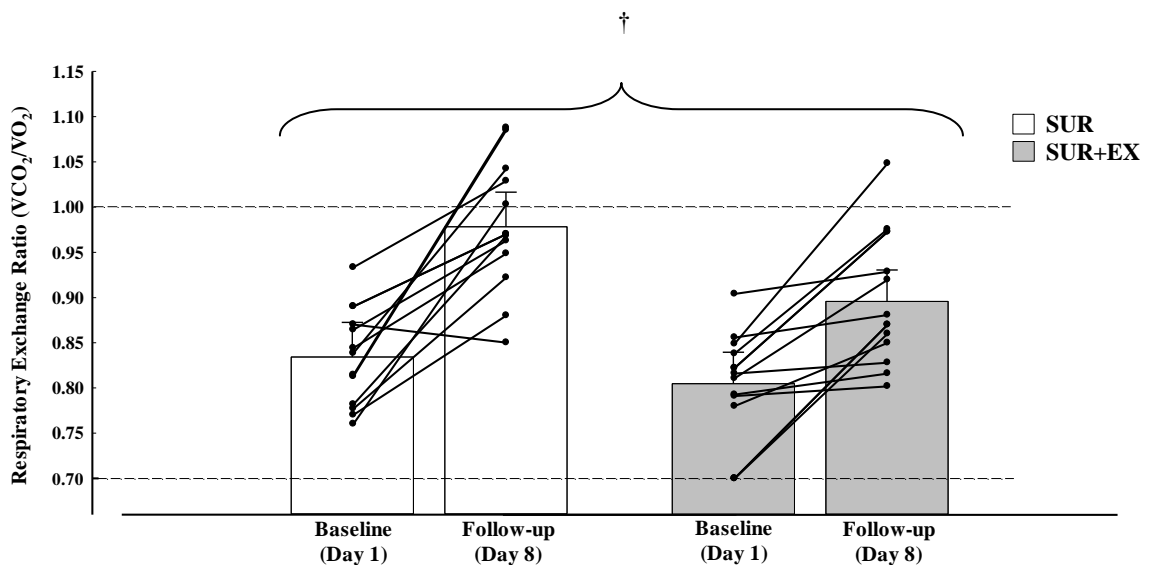


Figure 4.5: Respiratory Exchange Ratio at baseline and follow-up for the SUR group (n=13) and the SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=54.5$, $P<0.001$). Dotted lines denote the expected RER range based on carbohydrate and lipid oxidation only.

4.3.5 Anthropometric and physiological measures

Anthropometric and physiological measures pre and post-intervention are summarised in Table 4.5. Body mass (balance scales & DEXA), waist and hip circumference and lean mass (DEXA) significantly increased post-intervention in both groups ($P \leq 0.05$ baseline *versus* follow-up); this increase was different between groups (day \times group interaction; $F > 5.9$, $P \leq 0.05$). Fat mass (Bio-Impedance) significantly increased post-intervention in the SUR group ($P \leq 0.05$ baseline *versus* follow-up); this increase was different between groups (day \times group interaction; $F = 7.7$, $P = 0.01$). Fat mass (Skinfolds & DEXA), lean mass (Bio-Impedance), abdominal fat (DEXA) and systolic blood pressure significantly increased post-intervention in both groups ($P \leq 0.05$ baseline *versus* follow-up); there was a main effect of day ($F > 4.2$, $P \leq 0.05$) as shown in Table 4.5.

Table 4.5: Anthropometric and physiological characteristics measured before and after 7 days of overfeeding and reduced physical activity with (SUR+EX; n=12) or without (SUR; n=14) an exercise prescription. (n.b. for DEXA measurements n=11 & 10, respectively). Mean \pm SD. Change scores shown with 95 % confidence intervals. * $P \leq 0.05$ day \times group interaction. # $P \leq 0.05$ baseline *versus* follow-up. † main effect of day (i.e.: Day 1 vs. Day 8 both groups; $P \leq 0.05$).

	SUR			SUR+EX		
	Baseline	Follow-up	Δ (95 % CI)	Baseline	Follow-up	Δ (95 % CI)
Body Mass (kg; Scales)	75.5 \pm 8.9	78.2 \pm 9.2 #	2.7 (2.0 to 3.3)	78.2 \pm 11.0	79.9 \pm 11.3 #	1.6 (1.0 to 2.3) *
Body Mass (kg; DEXA)	71.8 \pm 7.8	74.6 \pm 8.0 #	2.8 (2.1 to 3.5)	79.0 \pm 10.6	80.7 \pm 10.8 #	1.7 (1.0 to 2.3) *
Waist Circumference (cm)	81.7 \pm 5.2	84.1 \pm 5.0 #	2.4 (1.6 to 3.2)	83.7 \pm 7.1	84.8 \pm 6.9 #	1.1 (0.6 to 1.6) *
Hip Circumference (cm)	97.0 \pm 6.0	98.7 \pm 6.0 #	1.7 (1.2 to 2.3)	98.6 \pm 5.5	99.1 \pm 5.4 #	0.5 (0.0 to 0.9) *
Fat Mass (kg; DEXA)	10.5 \pm 3.6	10.7 \pm 3.4	0.1 (-0.5 to 0.7) †	12.4 \pm 4.1	13.0 \pm 4.5	0.6 (0.1 to 1.1) †
Fat Mass (kg; Skinfolds)	11.0 \pm 3.6	11.8 \pm 3.6	0.7 (0.3 to 1.1) †	11.1 \pm 3.8	11.7 \pm 3.7	0.6 (0.4 to 0.9) †
Fat Mass (kg; Bio. Imp.)	11.8 \pm 4.0	13.1 \pm 4.5 #	1.4 (0.8 to 1.9)	13.8 \pm 4.7	14.1 \pm 5.0	0.3 (-0.3 to 1.0) *
Lean Mass (kg; DEXA)	58.2 \pm 5.8	60.8 \pm 6.4 #	2.6 (1.9 to 3.4)	63.4 \pm 7.2	64.5 \pm 7.0 #	1.0 (0.1 to 2.0) *
Lean Mass (kg; Bio. Imp.)	60.6 \pm 5.8	61.9 \pm 5.6	1.3 (0.7 to 1.9) †	61.2 \pm 6.8	62.5 \pm 6.8	1.3 (0.5 to 2.0) †
Abdominal Fat (g; DEXA)	900 \pm 416	932 \pm 360	32 (-56 to 119) †	1206 \pm 477	1261 \pm 479	55 (25 to 85) †
Systolic BP (mmHg)	122 \pm 10	128 \pm 8	6 (1 to 11) †	126 \pm 9	128 \pm 10	2 (-2 to 6) †
Diastolic BP (mmHg)	69 \pm 9	71 \pm 4	2 (-2 to 6)	70 \pm 7	70 \pm 7	0 (-4 to 4)

4.3.6 Insulin, C-peptide & Glucose responses to OGTT

Serum Insulin

The serum insulin concentrations in response to the OGTT were significantly greater at follow-up for the SUR group but not for the SUR+EX group as shown in Figure 4.6. There was a main effect of day ($P<0.001$) and a day \times time \times group interaction ($P=0.003$). The insulinaemic response following the OGTT was significantly greater at follow-up for the SUR group increasing their 2 h insulin iAUC. The 2 h insulin iAUC of the SUR+EX group was unaffected as shown in Figure 4.7. There was a main effect of day ($P=0.001$) and a day \times group interaction ($P=0.002$).

There was also a main effect of day ($P<0.001$) and a day \times group interaction ($P<0.001$) for the serum insulin peak concentration during the 2 h OGTT with the SUR group increasing their peak concentration while the serum insulin peak concentration for the SUR+EX group was unaffected as shown in Figure 4.8. The individual serum insulin peak time for the SUR group was unaffected (from 46 ± 20 to 45 ± 14 min) while serum insulin peaked earlier at follow-up for the SUR+EX group (from 48 ± 25 to 39 ± 14 min) but this was not statistically significant.

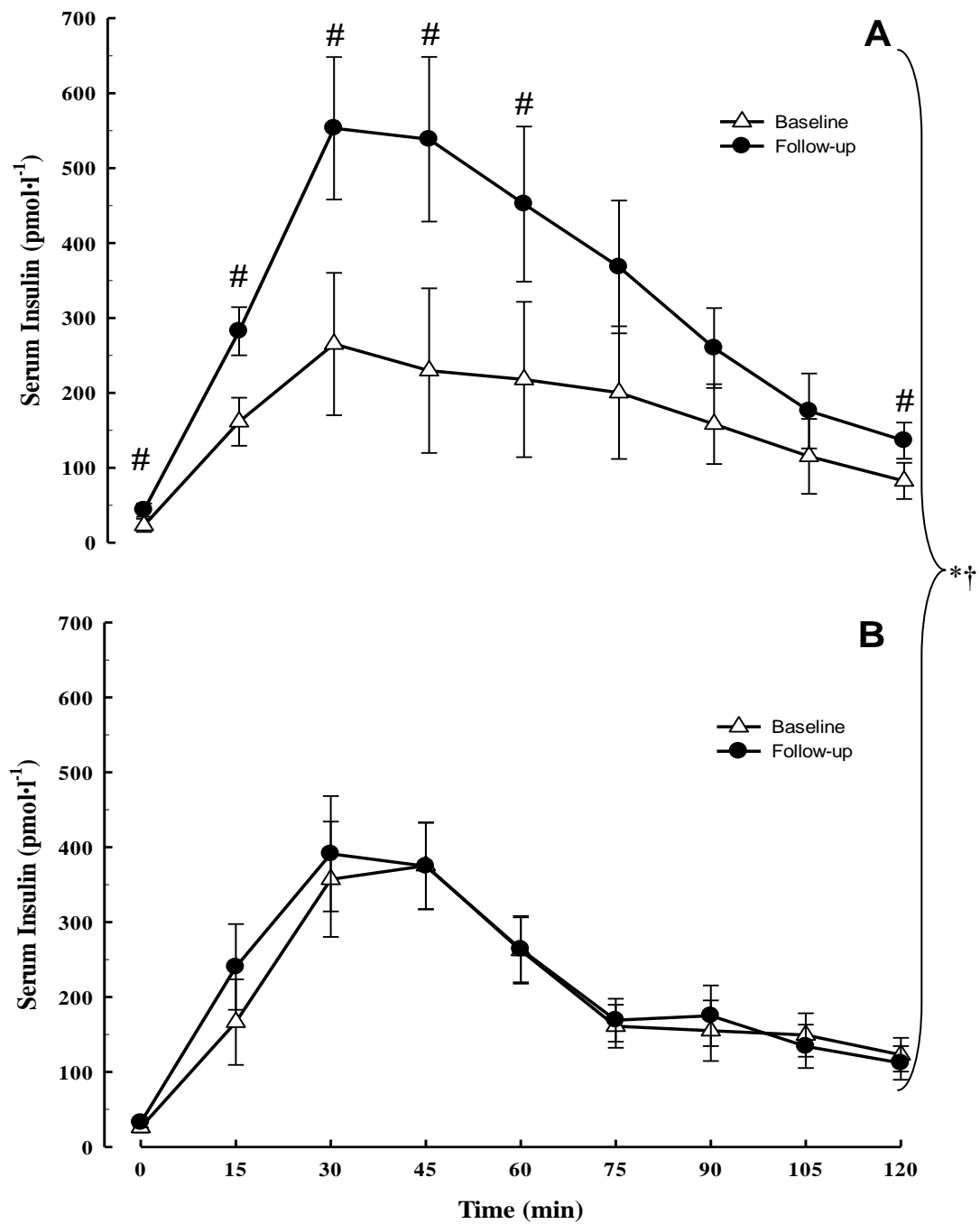


Figure 4.6: Serum insulin concentrations in response to the OGTT before and after a week of overfeeding and reduced physical activity (Panel A; SUR group) or after a week of overfeeding and reduced physical activity + exercise (Panel B; SUR+EX group). Values are means \pm nCI. * denotes a day \times time \times group interaction ($F=5.04$, $P=0.003$) # denotes values different pre-post ($P\leq 0.05$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=19.4$, $P<0.001$).

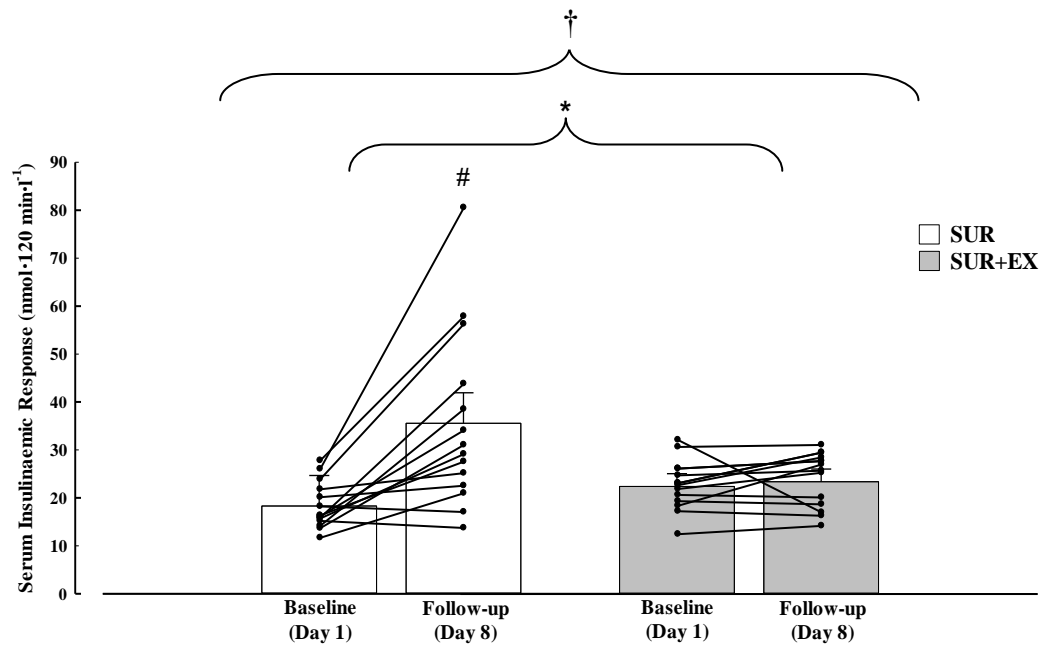


Figure 4.7: Insulinaemic responses during the 2 h OGTT for the SUR and SUR+EX group. Values are means \pm nCI. * denotes a dayxgroup interaction ($F=11.59$, $P=0.002$) # denotes values different pre-post within SUR group ($P=0.001$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=14.5$, $P=0.001$).

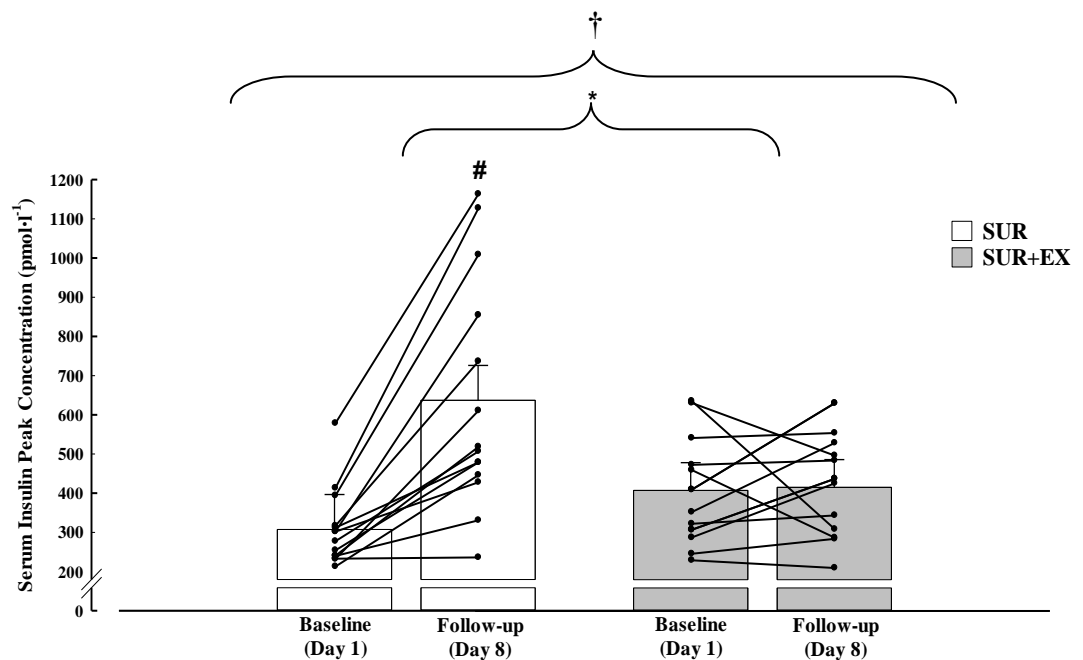


Figure 4.8: Serum Insulin peak concentration during the 2 h OGTT for the SUR and SUR+EX group. Values are means \pm nCI * denotes a dayxgroup interaction ($F=18.01$, $P<0.001$) # denotes values different pre-post within SUR group ($P<0.001$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=19.8$, $P<0.001$).

Serum C-peptide

The serum C-peptide concentrations in response to the OGTT were significantly greater at follow-up for the SUR group but not for the SUR+EX group as shown in Figure 4.9. There was a main effect of day ($P<0.001$) and a day \times time \times group interaction ($P=0.06$). C-peptide response following the OGTT was significantly greater at follow-up for the SUR group while the 2 h C-peptide iAUC of the SUR+EX group decreased as shown in Figure 4.10. There was a day \times group interaction ($P=0.002$).

The insulin data is also presented with $n=8$ in both group and with 5 time points during the OGTT (every 30 min) to enable comparison with the C-peptide data where $n=8$. The same subset of participants was analysed for both insulin and C-peptide.

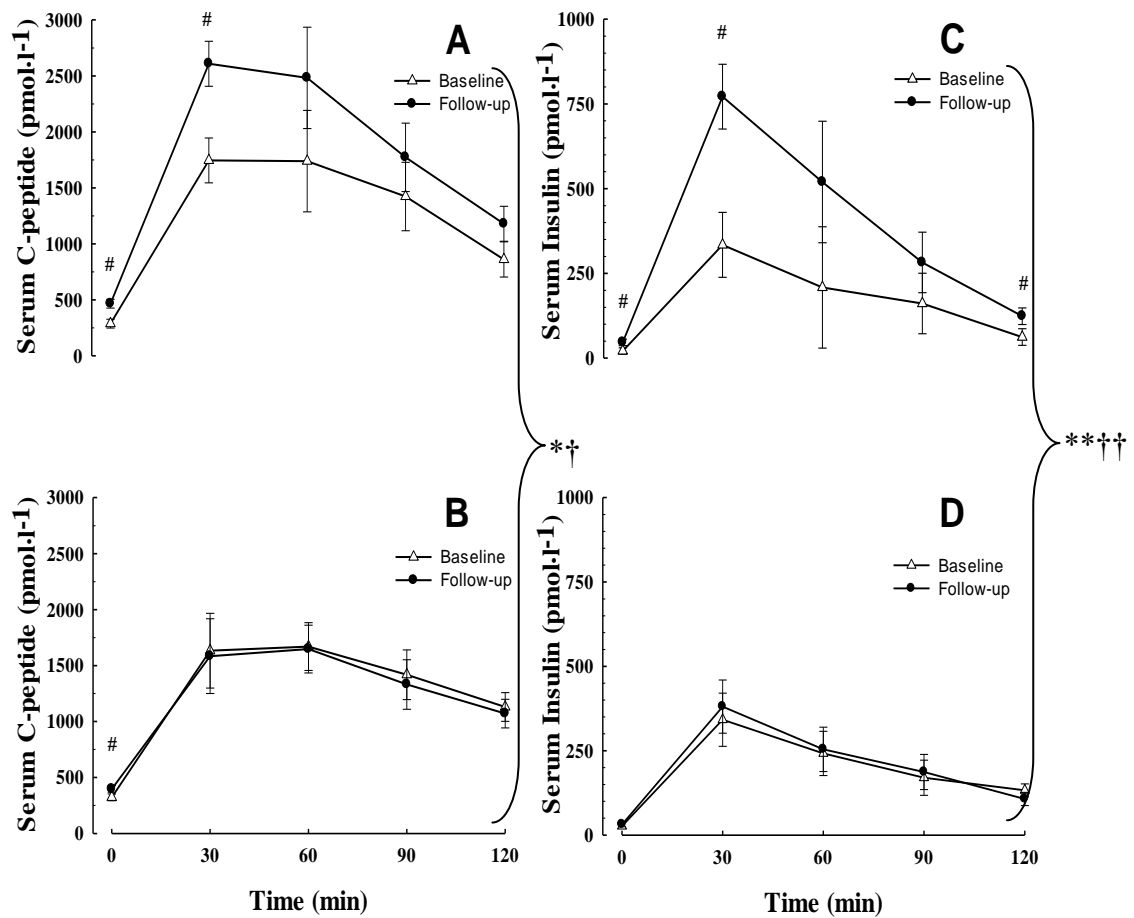


Figure 4.9: Serum C-peptide & insulin concentrations in response to the OGTT before and after a week of overfeeding and reduced physical activity (Panel A; C-peptide; SUR group, n=8), (Panel C; insulin; SUR group, n=8) or after a week of overfeeding and reduced physical activity + exercise (Panel B; C-peptide; SUR+EX group, n=8), (Panel D; insulin; SUR+EX group, n=8). Values are means \pm nCI * denotes a day \times time \times group interaction ($F=2.92$, $P=0.06$). ** denotes a day \times time \times group interaction ($F=6.63$, $P=0.003$) # denotes values different pre-post ($P\leq 0.05$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=20.2$, $P<0.001$) †† denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=27.4$, $P=0.001$).

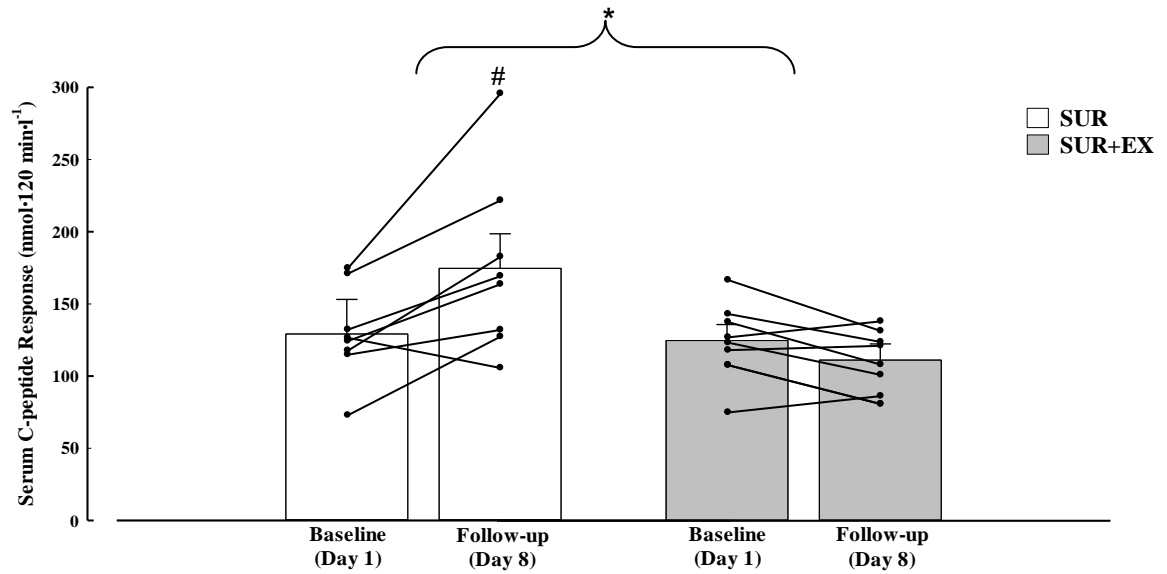


Figure 4.10: C-peptide responses during the 2 h OGTT for the SUR group (n=8) and the SUR+EX group (n=8). Values are means \pm nCI. # denotes values different pre-post within SUR group ($P=0.02$) * denotes a dayxgroup interaction ($F=13.89$, $P=0.002$).

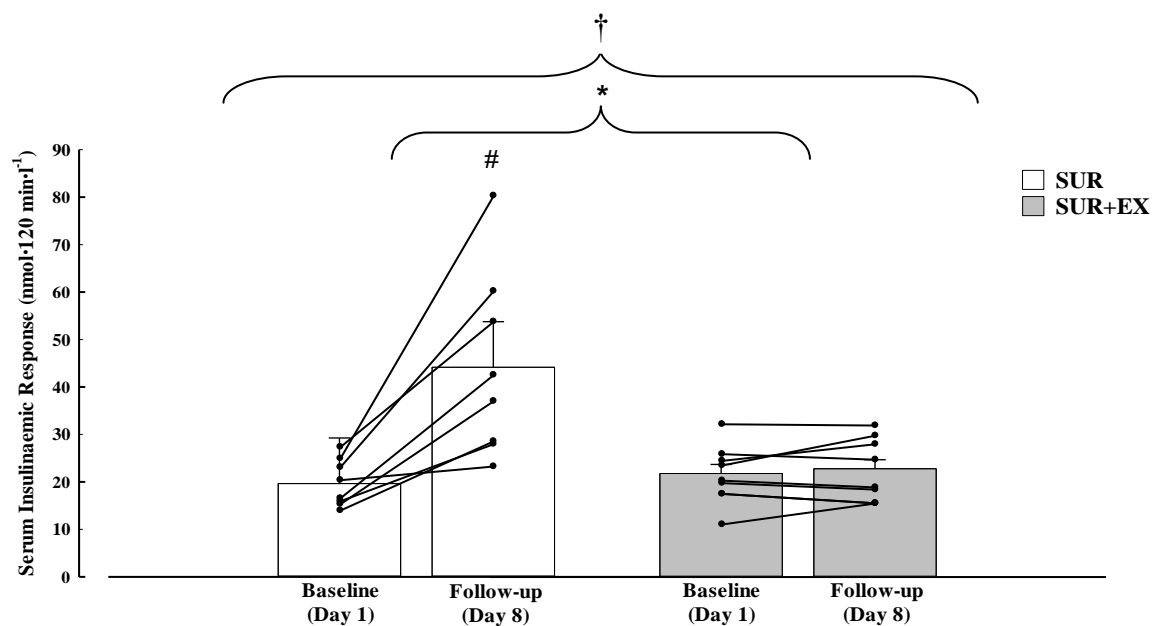


Figure 4.11: Insulinaemic responses during the 2 h OGTT for the SUR group (n=8) and the SUR+EX group (n=8). Values are means \pm nCI * denotes a dayxgroup interaction ($F=16.09$, $P=0.001$) # denotes values different pre-post within SUR group ($P=0.004$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=19.0$, $P=0.001$).

Plasma Glucose

The glycaemic response following the OGTT was unaffected in both groups as shown by Figure 4.12 & 4.13.

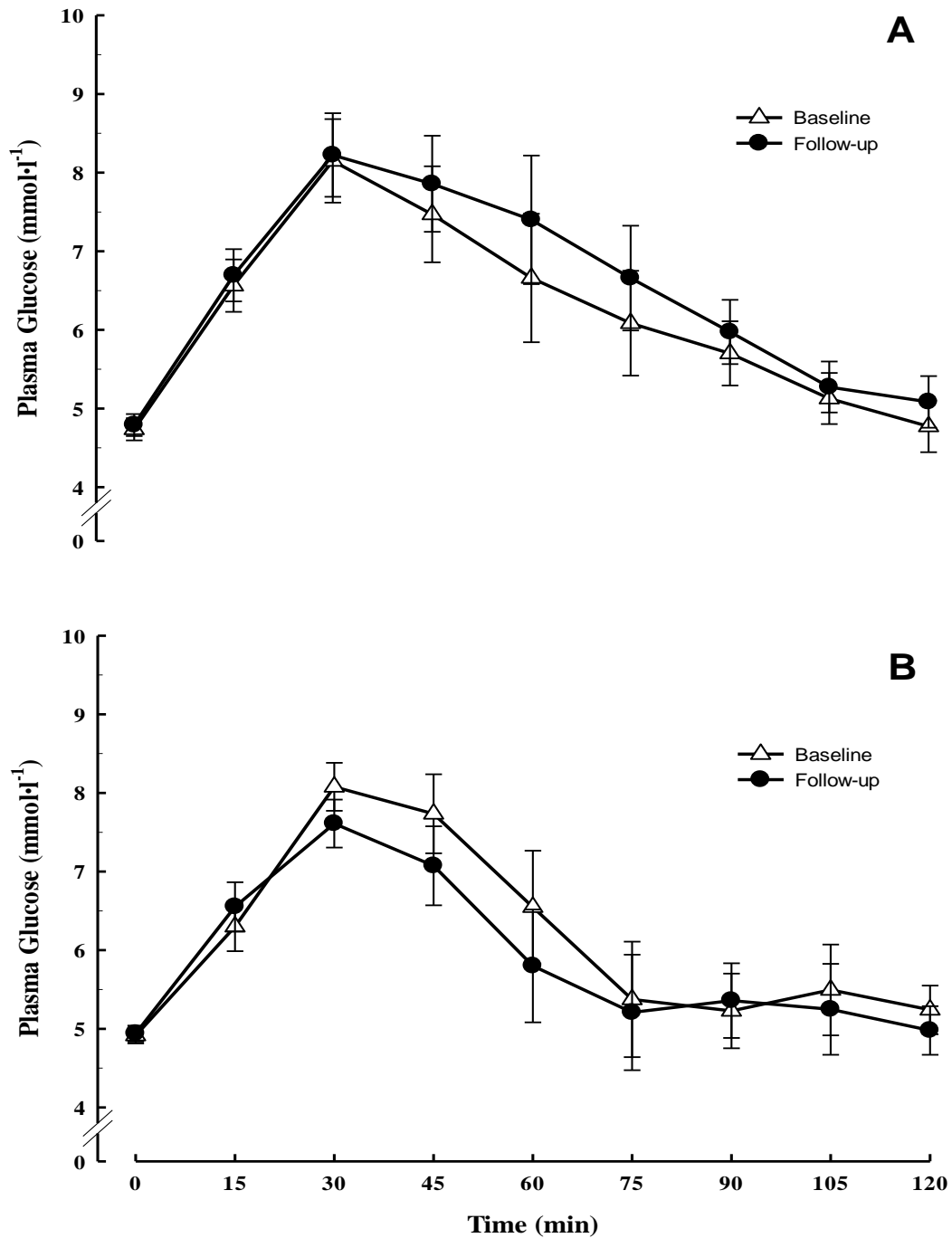


Figure 4.12: Plasma glucose concentrations in response to the OGTT before and after a week of overfeeding and reduced physical activity (Panel A; SUR group) or after a week of overfeeding and reduced physical activity + exercise (Panel B; SUR+EX group). Values are means \pm nCI.

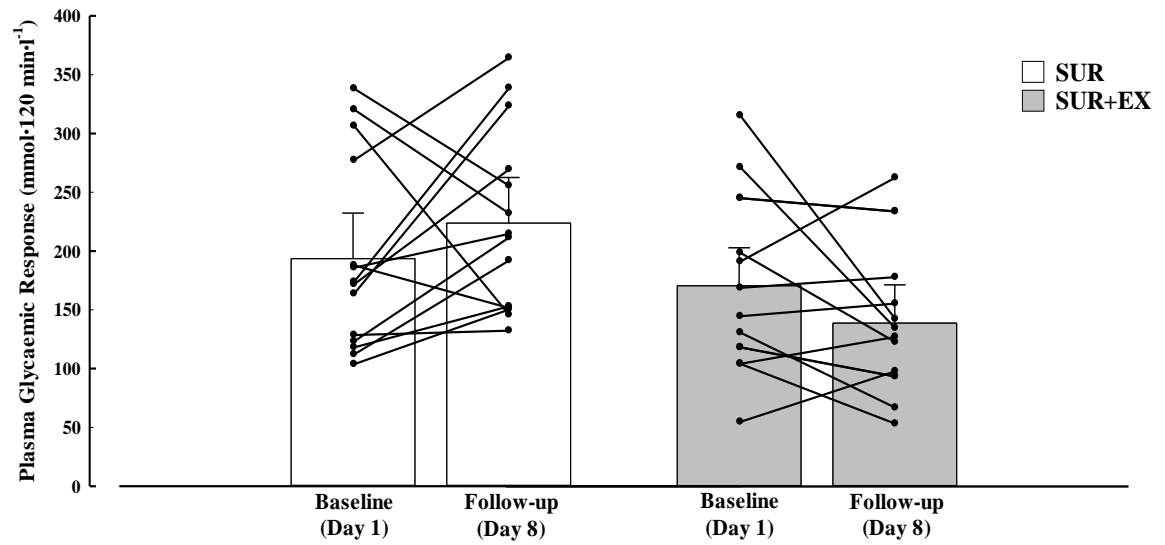


Figure 4.13: Glycaemic responses during the 2 h OGTT for the SUR and SUR+EX group. Values are means \pm nCI.

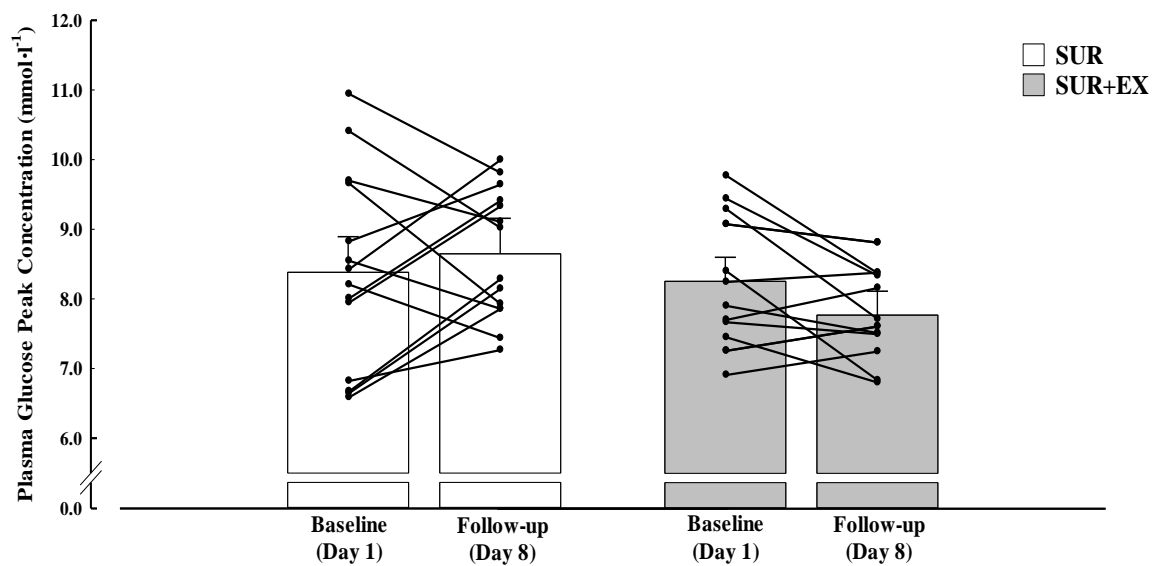


Figure 4.14: Plasma glucose peak concentration during the 2 h OGTT for the SUR and SUR+EX group. Values are means \pm nCI.

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) increased at follow-up in both groups; there was a day \times group trend ($F=3.5$, $P=0.07$) and a main effect of day ($F=14.3$, $P=0.001$). The Homeostasis Model Assessment of β -cell function (HOMA- β) increased at follow-up in the SUR group ($P\leq 0.05$ pre-post within SUR group); there was a day \times group interaction ($F=4.9$, $P=0.04$). The Matsuda Index or Composite Index (ISI comp) decreased at follow-up in the SUR group ($P\leq 0.05$ pre-post within SUR group); there was a day \times group interaction ($F=4.9$, $P=0.04$) as shown in Table 4.6.

Table 4.6: HOMA-IR, HOMA- β and Composite Index values at baseline and follow-up. Mean \pm SD. Change scores shown with 95 % confidence intervals. * $P \leq 0.05$ day \times group interaction # $P \leq 0.05$ baseline *versus* follow-up † main effect of day (i.e.: Day 1 *vs.* Day 8 both groups; $P=0.001$).

	SUR			SUR+EX		
	Baseline	Follow-up	Δ (95 % CI)	Baseline	Follow-up	Δ (95 % CI)
HOMA-IR	0.79 ± 0.67	1.54 ± 0.85	0.75 (0.30 to 1.20) †	0.95 ± 0.58	1.20 ± 0.61	0.25 (-0.08 to 0.59) †
HOMA-β (%)	63 ± 53	119 ± 73 #	57 (23 to 91)	60 ± 33	74 ± 33	14 (-9 to 37) *
Insulin Sensitivity Index (comp)	11.9 ± 4.2	6.2 ± 2.5 #	5.6 (3.4 to 7.8)	10.8 ± 5.9	8.9 ± 4.2	1.8 (-1.4 to 5.0) *

4.3.7 Fasted Blood measurements

Serum adiponectin concentrations significantly increased at follow-up within the SUR and SUR+EX group; there was a main effect of day ($P<0.001$) and a day \times group interaction ($P\leq 0.05$) as shown by Figure 4.15. Serum leptin concentrations significantly increased at follow-up within the SUR and SUR+EX group; there was a main effect of day ($P<0.001$) as shown by Figure 4.16.

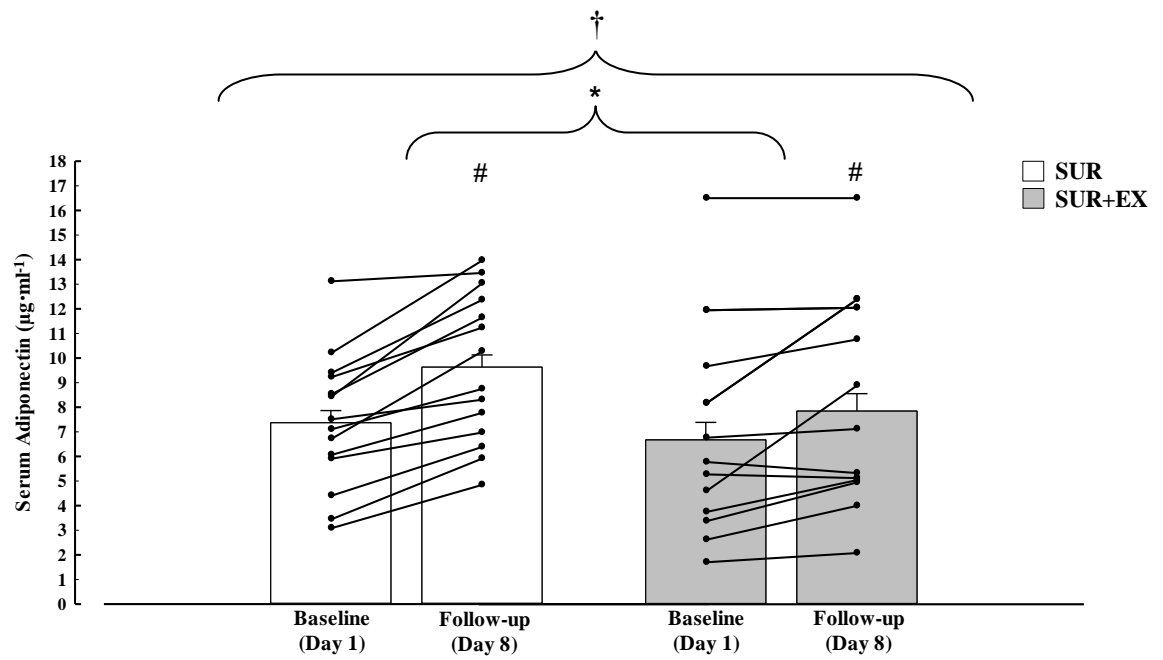


Figure 4.15: Serum Adiponectin concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI * denotes a dayxgroup interaction ($F=4.17$, $P\leq 0.05$) # denotes values different pre-post within SUR and SUR+EX group ($P<0.001$ & $P=0.03$, respectively) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=39.5$, $P<0.001$).

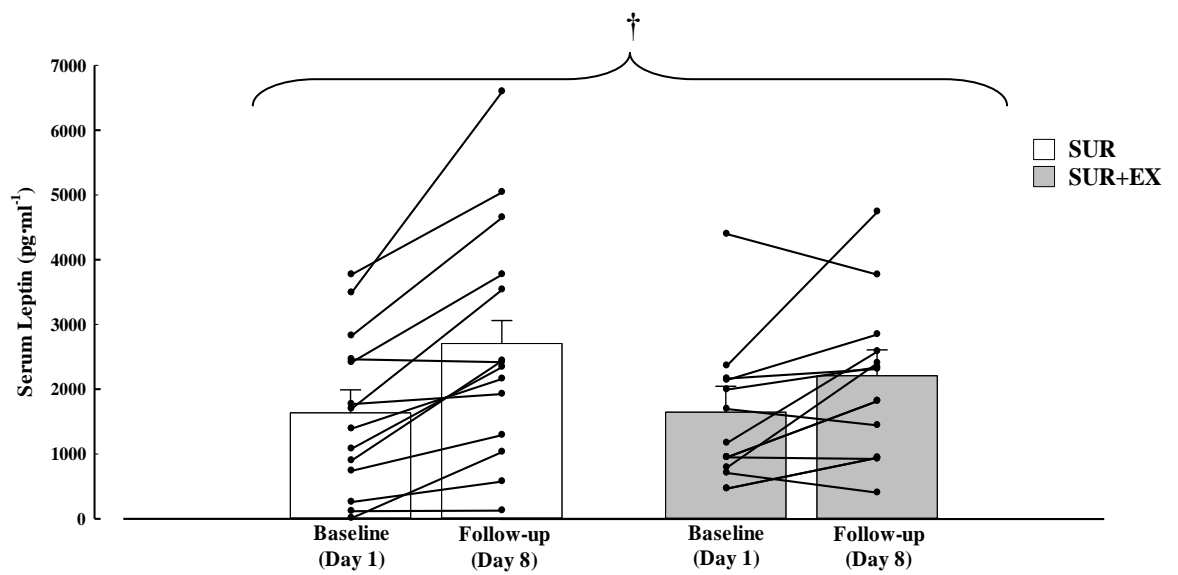


Figure 4.16: Serum Leptin concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=22.4$, $P<0.001$).

Total serum cholesterol concentrations significantly increased at follow-up in the SUR group while they remained unchanged in the SUR+EX group; there was a main effect of day ($P=0.005$) and a day \times group interaction ($P=0.03$) as shown by Figure 4.17. HDL cholesterol concentrations increased in both groups at follow-up; there was a main effect of day ($P=0.002$) as shown by Figure 4.18. LDL cholesterol concentrations increased at follow-up within the SUR group but decreased within the SUR+EX group, there was a main effect of day ($P=0.002$) and a day \times group interaction ($P\leq 0.05$) as shown by Figure 4.19. Triglyceride concentrations increased in both groups at follow-up; there was a main effect of day ($P=0.003$) as shown by Figure 4.20. Non-esterified fatty acids concentrations decreased at follow-up; there was a main effect of day ($P<0.001$) as shown by Figure 4.21. Serum ALT activity (Figure 4.22) increased in both groups at follow-up; there was a main effect of day ($P=0.003$).

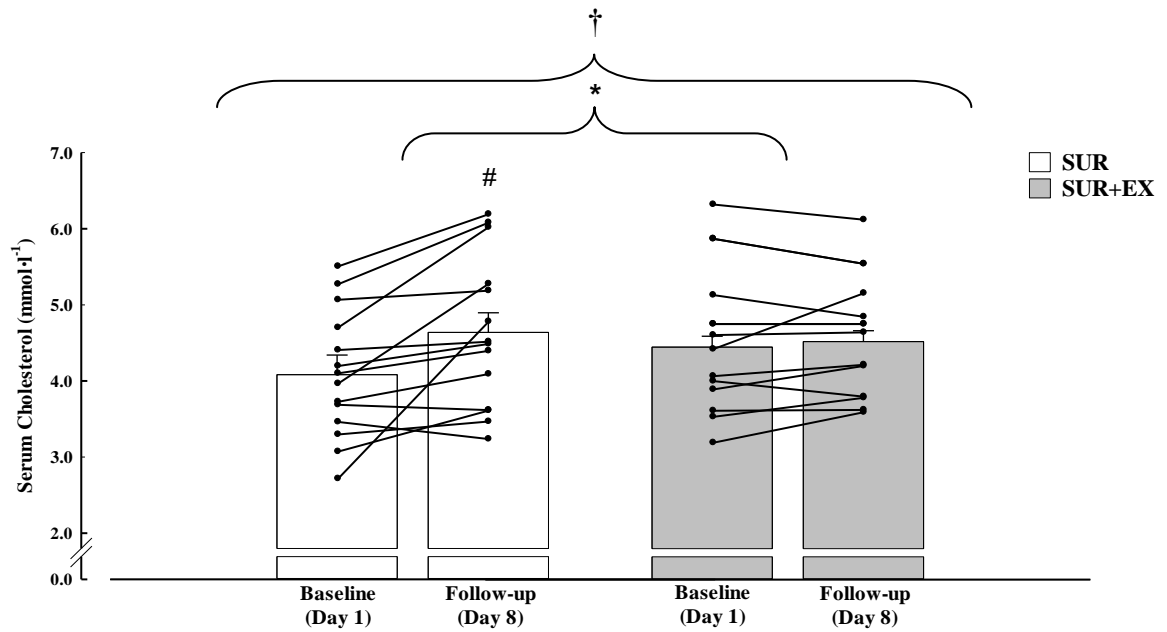


Figure 4.17: Total serum cholesterol concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. * denotes a day x group interaction ($F=5.73$, $P=0.03$) # denotes values different pre-post within SUR group ($P=0.006$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=9.8$, $P=0.005$).

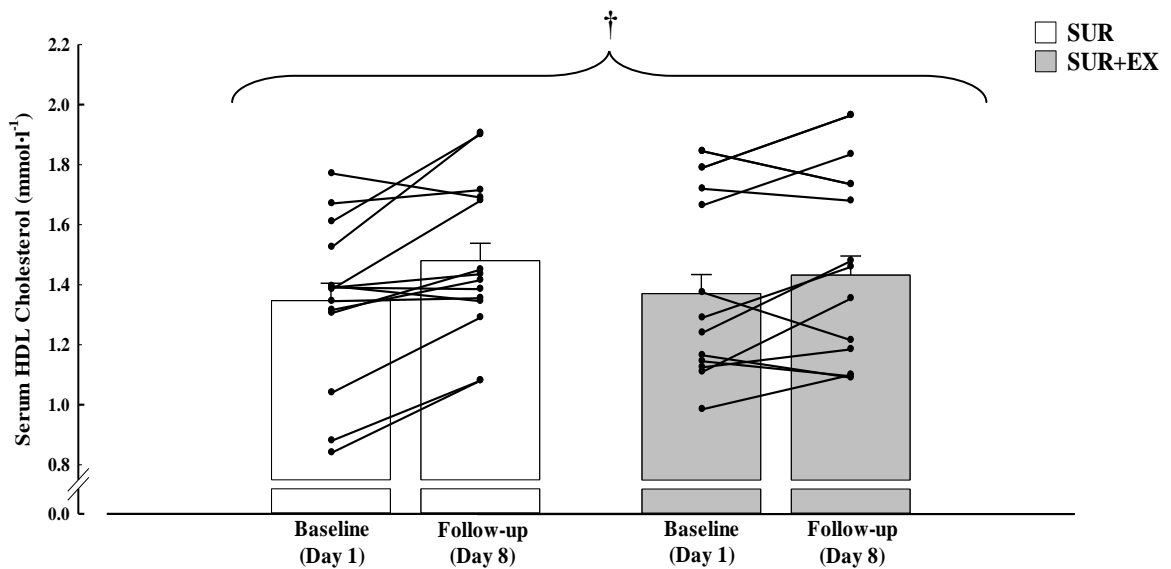


Figure 4.18: Serum HDL cholesterol concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=11.9$, $P=0.002$).

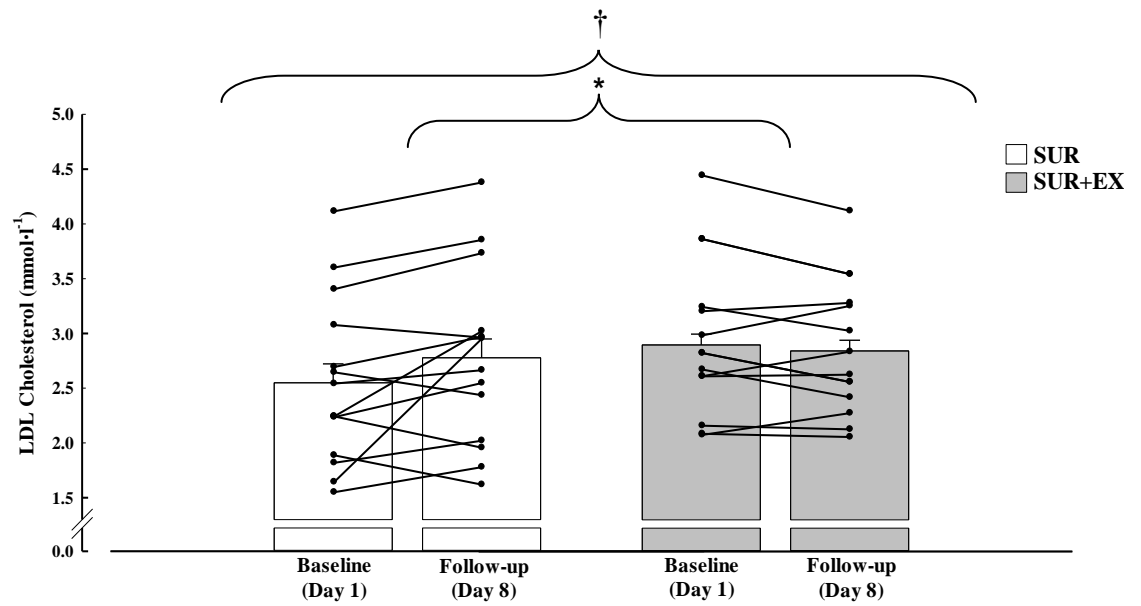


Figure 4.19: LDL cholesterol at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. * denotes a day \times group interaction ($F=4.34$, $P\leq 0.05$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=11.4$, $P=0.002$).

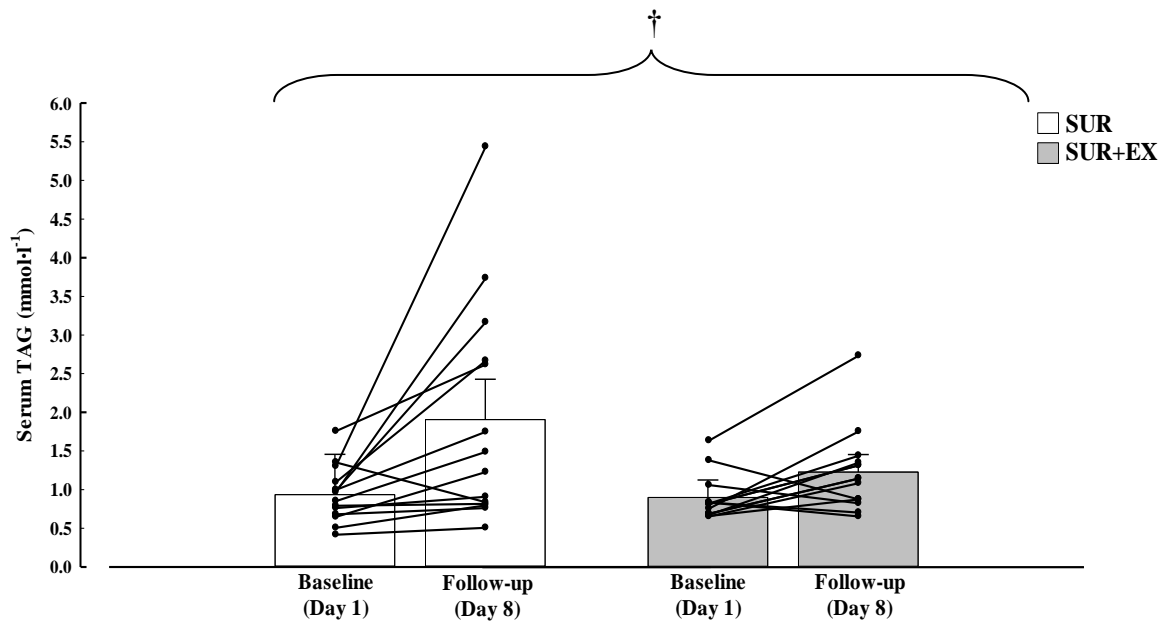


Figure 4.20: Serum triglycerides (TAG) concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=11.0$, $P=0.003$).

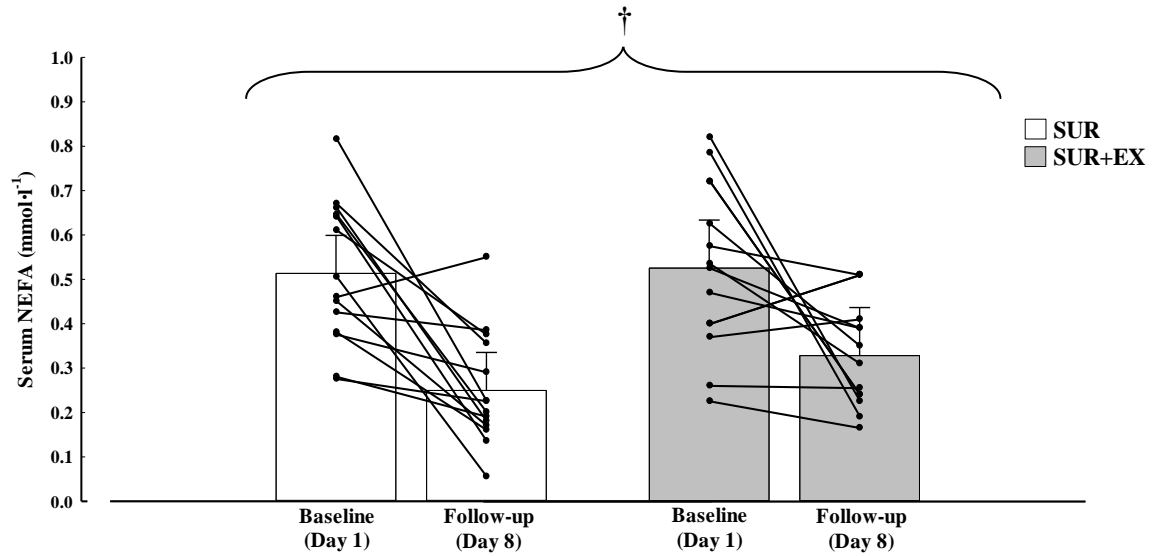


Figure 4.21: Serum NEFA concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=27.3$, $P<0.001$).

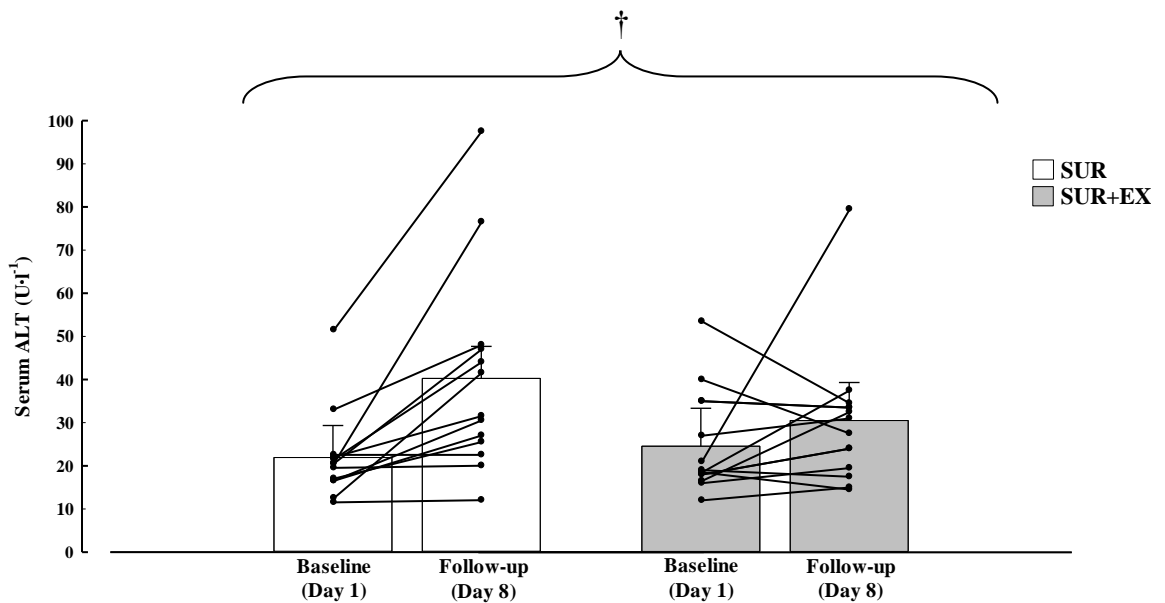


Figure 4.22: Serum ALT activity at baseline and follow-up for the SUR group ($n=13$) and the SUR+EX group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=10.8$, $P=0.003$).

Whole blood white blood cell count significantly increased at follow-up in the SUR group while it remained unchanged in the SUR+EX group; there was a main effect of day ($P=0.01$) and a day \times group interaction ($P=0.03$) as shown by Figure 4.23. CRP concentrations were not affected as shown by Figure 4.24. Serum IL-6 concentrations (Figure 4.25) were also unaffected.

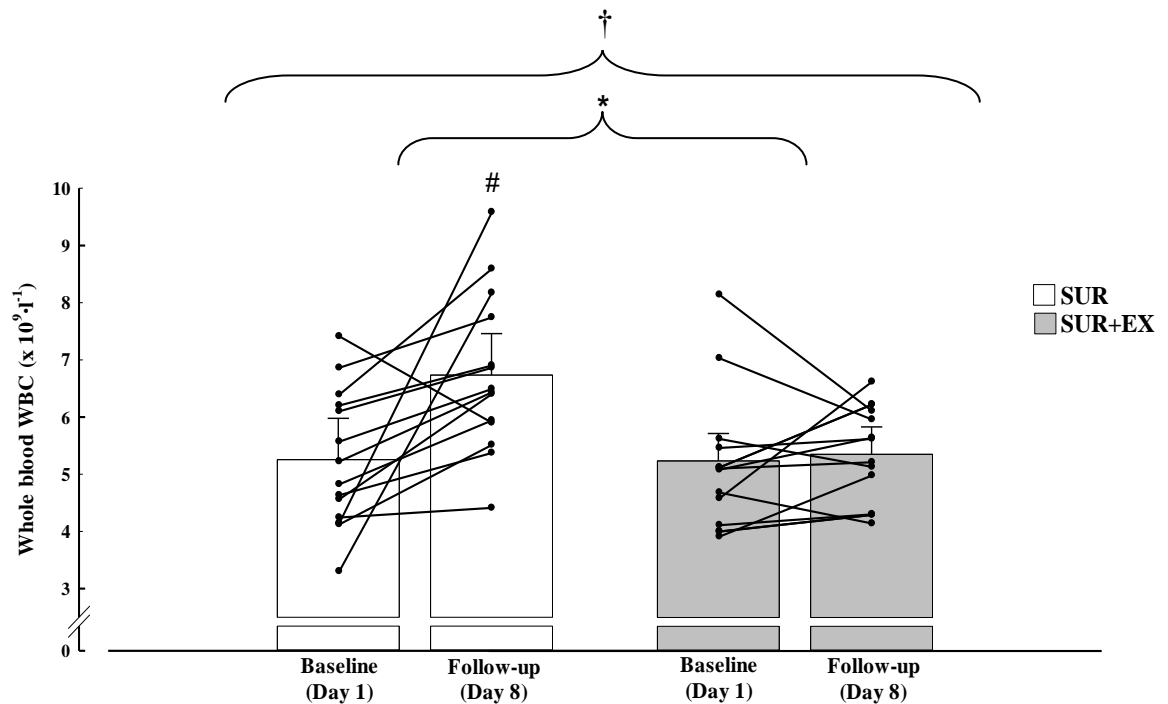


Figure 4.23: Whole blood white blood cell count at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI. * denotes a day \times group interaction ($F=5.38$, $P=0.03$) # denotes values different pre-post within SUR group ($P=0.008$) † denotes a main effect of day (i.e.: Day 1 vs. Day 8 both groups; $F=7.4$, $P=0.01$).

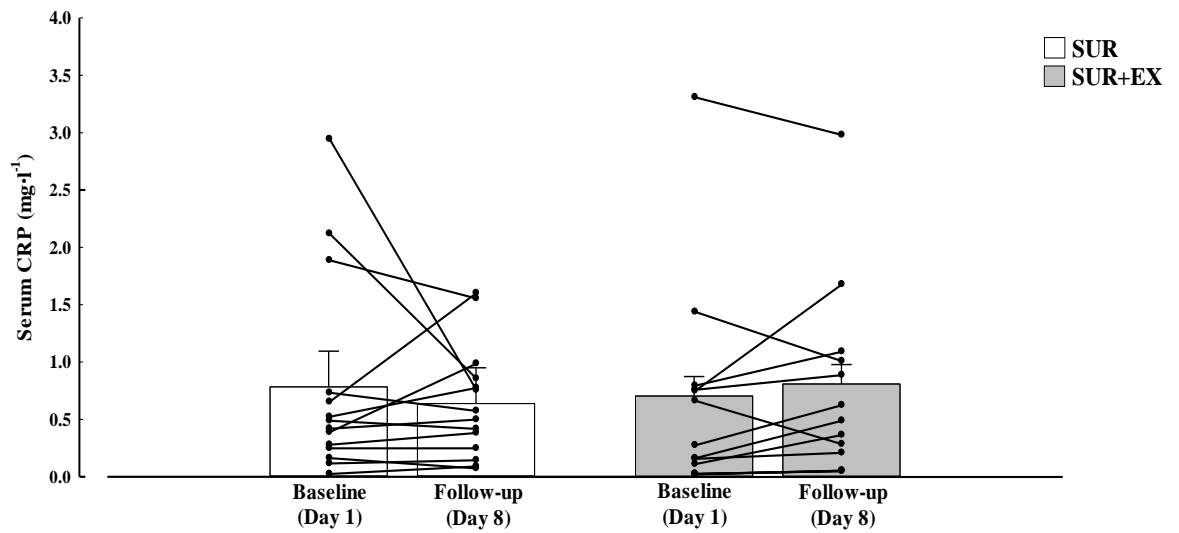


Figure 4.24: Serum CRP concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI.

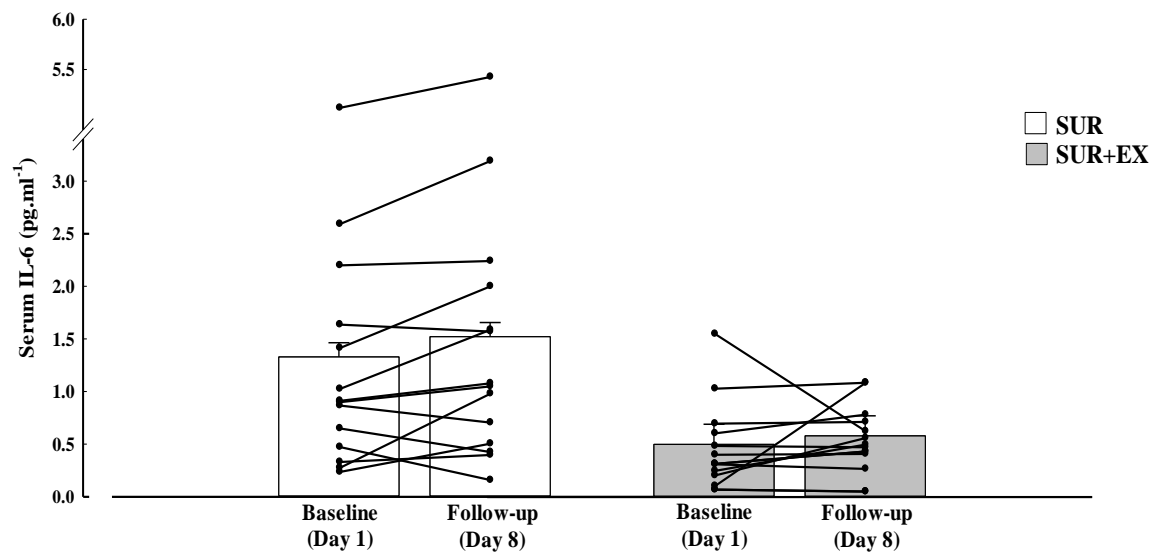


Figure 4.25: Serum IL-6 concentrations at baseline and follow-up for the SUR and SUR+EX group. Values are means \pm nCI.

Gene/Protein**4.3.8 Adipose tissue Gene Expression**

Expression of SREBP1c, FAS and GLUT4 was significantly up-regulated in the SUR group ($P \leq 0.01$ baseline *versus* follow-up); there was a main effect of day ($F > 9.8$, $P < 0.01$) and a day \times group interaction ($F > 5.2$, $P \leq 0.05$). Expression of IRS2 was significantly down-regulated in the SUR group ($P < 0.001$ baseline *versus* follow-up); there was a main effect of day ($F = 11.8$, $P = 0.003$) and a day \times group interaction ($F = 17.8$, $P < 0.001$). Expression of VISFATIN was significantly down-regulated in the SUR group ($P = 0.02$ baseline *versus* follow-up); there was a day \times group interaction ($F = 6.2$, $P = 0.02$). Expression of PDK4 and HSL was significantly down-regulated in the SUR and SUR+EX groups ($P \leq 0.05$ baseline *versus* follow-up); there was a main effect of day ($F > 25.4$, $P < 0.001$) and a day \times group interaction ($F > 5.3$, $P \leq 0.05$). There was a main effect of day in the expression changes of ADIPONECTIN, LEPTIN, PPAR γ , AMPK and Apelin ($F > 5.4$, $P \leq 0.05$). Individuals with one or both samples outside the detectable limit ($Ct > 35$) were excluded from the analysis. Four genes were expressed at very low levels; TNF α was undetectable in 9 % of samples; IL-18 was undetectable in 32 % of samples; IL-6 was undetectable in 39 % of samples and Apelin was undetectable in 50 % of samples. Gene expression data is presented below (Figure 4.26). Enough RNA could not be obtained from some smaller biopsies to carry out gene expression analysis on those samples, as a result $n=10$ in the SUR group and $n=12$ in the SUR+EX group.

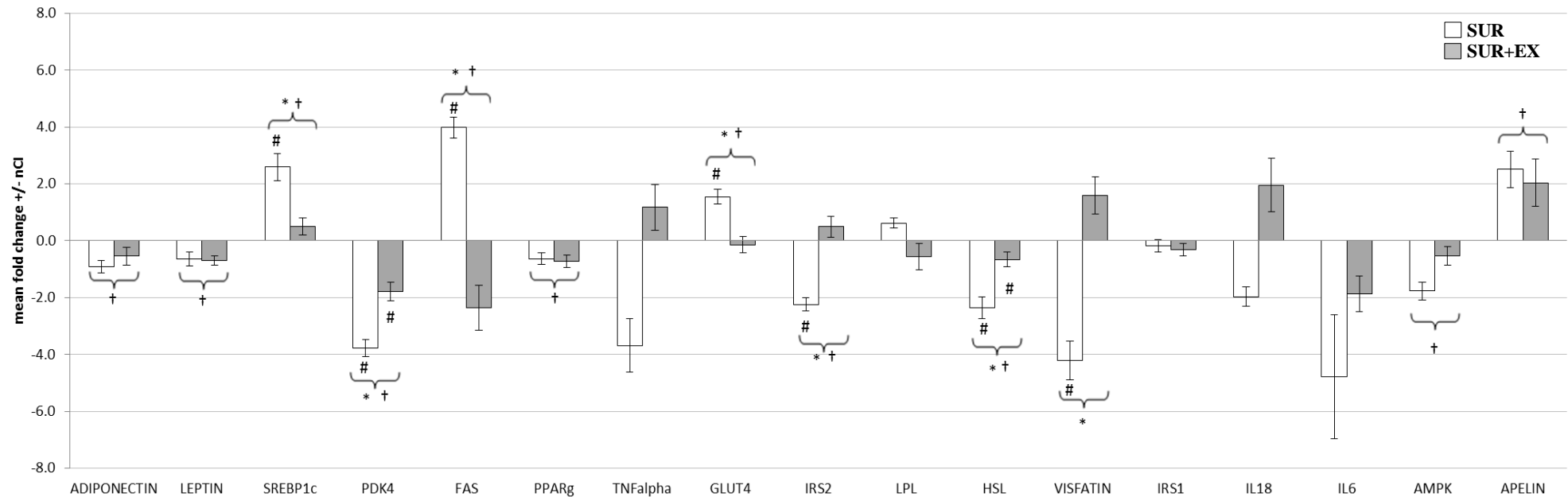


Figure 4.26 : Mean fold change in the expression of several key genes measured in adipose tissue at baseline and follow-up for the SUR group (n=10) and the SUR+EX group (n=12). Data normalised to PPIA= Peptidylprolyl isomerase A. SREBP-1c= Sterol regulatory element binding protein 1c, PDK4= Pyruvate dehydrogenase kinase isozyme 4, FAS= Fatty acid synthase, PPAR γ = Peroxisome proliferator-activated receptor gamma, TNF α = Tumor necrosis factor alpha (SUR, n=9; SUR+EX, n=10), GLUT4= Glucose transporter type 4, IRS2= Insulin receptor substrate 2, LPL= Lipoprotein lipase, HSL= Hormone-sensitive lipase. IRS1= Insulin receptor substrate 1, IL18= Interleukin-18 (SUR, n=6; SUR+EX, n=8), IL6= Interleukin-6 (SUR, n=4; SUR+EX, n=6), AMPK= AMP-activated kinase (SUR, n=5; SUR+EX, n=6). Values are means \pm nCI. * $P \leq 0.05$ day \times group interaction. # $P \leq 0.05$ baseline *versus* follow-up. † main effect of day (i.e.: Day 1 vs. Day 8 both groups; $P \leq 0.05$).

4.3.9 Western Blots Analysis

The protein ratio between pAMPK and AMPK was significantly down-regulated in the SUR group ($P=0.001$ baseline *versus* follow-up); there was a day \times group interaction ($F=11.3$, $P=0.005$). None of the other proteins measured (GLUT4, pAMPK, AMPK, pAKT, AKT1 & 2) or ratios (pAKT/AKT1 & pAKT/AKT2) were significantly impacted by the overfeeding and restricted physical activity model. Protein expression data is presented below (Figure 4.27). A subset of samples representative of the overall group (mean response to the OGTT was very similar to the group mean) were analysed (SUR group, $n=7$; SUR+EX, $n=8$).

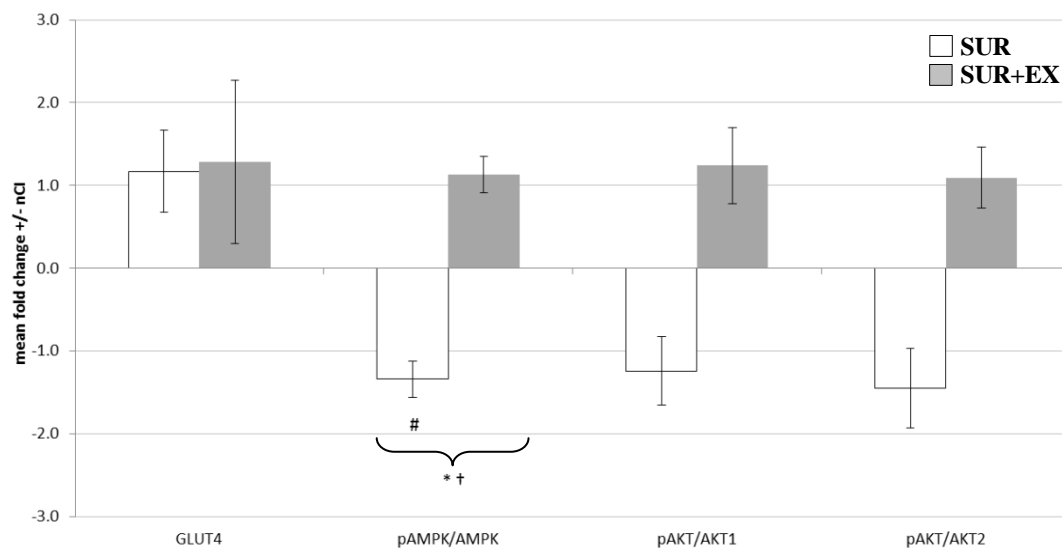


Figure 4.27 : Mean fold change in the expression of several key proteins measured in adipose tissue at baseline and follow-up for the SUR group ($n=7$) and the SUR+EX group ($n=8$). Data normalised to GAPDH= Glyceraldehyde 3-phosphate dehydrogenase. GLUT4= Glucose transporter type 4, pAMPK= phospho 5' AMP-activated protein kinase, AMPK= 5' AMP-activated protein kinase, pAKT= phospho Protein Kinase B, AKT1= RAC-alpha serine/threonine-protein kinase, AKT2= RAC-beta serine/threonine-protein kinase. Values are means \pm nCI. * $P \leq 0.05$ day \times group interaction. # $P \leq 0.05$ baseline *versus* follow-up. † main effect of day (i.e.: Day 1 *vs.* Day 8 both groups; $P \leq 0.05$).

4.4 Discussion

The combination of short-term overfeeding and reduced physical activity had a dramatic impact on insulin sensitivity and adipose tissue gene expression. We demonstrated that 7 days of positive energy balance induced a state of marked insulin insensitivity in healthy individuals, with significantly altered expression patterns of several key genes and proteins within adipose tissue involved in nutritional homeostasis, metabolism and insulin action. Notably, the inclusion of a daily vigorous-intensity exercise bout largely prevented these changes from taking place independent of any net effect on energy imbalance.

Combining reduced physical activity with overfeeding represents an experimental model to investigate the benefits of exercise independent of energy balance. We successfully maintained a comparable energy surplus between the SUR and SUR+EX groups. Importantly, the SUR+EX group expended ~700 kcal through daily exercise which was accounted for in the prescribed overfeeding. The SUR group experienced an increase in fasted insulin concentrations and a ~2-fold increase in the insulinaemic response to the OGTT whereas there was no such change in SUR+EX group. The greater insulin response to the OGTT reflects greater insulin secretion as indicated by the C-peptide response to the OGTT which provides a more accurate assessment of β -cell function than peripheral insulin levels as it overcomes the issue of hepatic insulin clearance (Cobelli *et al.*, 2007). Brief periods of positive energy balance have been shown to lead to impaired insulin sensitivity whether this is achieved through decreased physical activity (Vukovich *et al.*, 1996; Arciero *et al.*, 1998; Hamburg *et al.*, 2007; Sonne *et al.*, 2010), through overfeeding (Wang *et al.*, 2001; Cornier *et al.*, 2006; Brons *et al.*, 2009) or both (Knudsen *et al.*, 2012; Hagobian and Braun, 2006). Hagobian and Braun (2006) showed that a single 60-minute bout of exercise performed after 3 days of overfeeding and detraining partially *restored* the insulinaemic response to feeding. To our knowledge, the present study is the first to demonstrate that metabolic dysfunction resulting from a profound short term energy surplus can be mostly prevented by concurrent daily vigorous-intensity exercise.

Given that energy surplus is a major factor leading to obesity and resultant metabolic disturbances, it is surprising that so few studies have focused on changes in the expression patterns of key genes in adipose tissue during a bout of energy surplus in healthy individuals considering it is a major site for energy storage (Shea *et al.*, 2009; Alligier *et al.*, 2012). This is in contrast to the large amount of research that has been dedicated to investigating the development of insulin resistance within skeletal muscle (Vukovich *et al.*, 1996; Majer *et al.*, 1998; Schrauwen-Hinderling *et al.*, 2005; Krogh-Madsen *et al.*, 2006; Steinberg *et al.*, 2006; Lessard *et al.*, 2007; Plomgaard *et al.*, 2007; Tsintzas *et al.*, 2007; Alibegovic *et al.*, 2010; Jensen *et al.*, 2006, 2011). We elected to focus on expression changes within adipose tissue and seventeen genes involved in a variety of biological processes such as energy homeostasis (AMPK, LEPTIN, APELIN), glucose metabolism (IRS1&2, PDK4, GLUT 4, ADIPONECTIN), lipid metabolism (ADIPONECTIN, SREBP1c, FAS, PPAR γ , LPL, HSL, VISFATIN) and inflammatory pathways (TNF α , IL18, IL6) were selected. The overfeeding and reduced physical activity protocol adopted here dramatically impacted on the expression of these genes. Seven out of seventeen tested genes were differentially-expressed between the two groups. Crucially, the addition of a daily vigorous-intensity exercise bout mostly prevented alterations to the expression patterns of these genes independent of any net effect on energy imbalance.

Few studies have examined the impact of overfeeding and/or reduced physical activity on adipose tissue and this is surprising given its role in energy storage and metabolic control. AMPK plays a role in cellular energy homeostasis and increases glucose transport and fatty acid oxidation while switching off other pathways such as lipogenesis and gluconeogenesis. In the current study, AMPK mRNA in adipose tissue was down-regulated in both groups, more so in the SUR group. This was confirmed at the protein level and the ratio between pAMPK and AMPK was significantly down-regulated in the SUR group. This would be likely to reduce glucose uptake plus decrease fatty acid oxidation and both fasting and exercise have been shown to activate AMPK in adipose tissue (Daval *et al.*, 2006). Recently, AMPK has become a potential therapeutic target for the treatment of obesity and Type 2 Diabetes (Pedersen, 2007) and thus it is

particularly noteworthy that exercise prevented the changes to AMPK in adipose tissue even in the context of a profound energy surplus.

Expression of PDK4 in adipose tissue decreased in both groups but more so in the SUR group, highlighting a switch of oxidative fuel from fatty acids to glucose. PDK4 is a mitochondrial protein that is a member of the pyruvate dehydrogenase kinase family, a group of enzymes that inhibit the pyruvate dehydrogenase complex (PDC) by phosphorylating one of its subunits (Rowles *et al.*, 1996). Expression of this gene is regulated by glucocorticoids, retinoic acid and insulin (Kwon & Harris, 2004). Our data support the role of insulin since there was an inverse correlation between changes in insulin iAUC and changes in the expression of PDK4 ($r=-0.62$; $P=0.002$). There was also an inverse correlation between changes in insulin iAUC and changes in the expression of IRS2 ($r=-0.56$; $P=0.007$). IRS2 mRNA was down-regulated in the SUR group in response to the intervention but was not affected in the SUR+EX group. IRS2 is a cytoplasmic protein that mediates the effects of insulin and various cytokines (Sun *et al.*, 1995). The changes in PDK4 and IRS2 reported here are in agreement with transcriptome analysis conducted on abdominal subcutaneous fat samples from lean and obese men following short-term overfeeding (Shea *et al.*, 2009). We suggest that these changes within adipose tissue may be a secondary response to the marked hyperinsulinaemia induced by overfeeding.

SREBP-1c is a transcription factor that regulates the expression of the lipogenic enzymes FAS (Minehira *et al.*, 2003). Overfeeding and reduced activity significantly increased the expression of both SREBP-1c and FAS transcripts in the SUR group and changes in SREBP-1c and FAS were correlated ($r=0.63$; $P=0.002$). Minehira *et al.* (2003) made a similar finding in humans using a carbohydrate overfeeding protocol. Our participants consumed a diet rich in carbohydrates ($\sim 700\text{g}\cdot\text{day}^{-1}$) during the intervention and so it is likely that this would have rapidly saturated liver and muscle glycogen stores. The SUR group had little capacity for carbohydrate oxidation as a result of restricted physical activity thermogenesis and thus it is unsurprising that SREBP-1c and FAS are up-regulated in the SUR group as SREBP-1c has been linked with de novo

lipogenesis (DNL) which has been shown to take place in adipose tissue (Strawford *et al.*, 2004; Roberts *et al.*, 2009; Collins *et al.*, 2011); thus providing a route for disposal of excess glucose. Our calculations suggest that the increase in RMR in the SUR group may be explained by DNL; by removing the estimated energy cost of this process (Acheson *et al.*, 1988), RMR at follow-up would be similar in each group ($8112 \pm 1173 \text{ kJ}\cdot\text{day}^{-1}$ and $8166 \pm 1135 \text{ kJ}\cdot\text{day}^{-1}$ in SUR and SUR+EX groups, respectively). Collectively, our results indicate that combined overfeeding and reduced physical activity favours net lipogenesis (\uparrow SREBP-1c, \uparrow FAS, \downarrow HSL) but not when the same energy surplus occurs alongside daily vigorous intensity exercise.

Leptin is a hormone that is predominantly expressed in the adipose tissue. Our results suggest that leptin is regulated at the post-transcriptional level as mRNA levels are not affected by the intervention while serum levels increased 1.7-fold in the SUR group and 1.3-fold in the SUR+EX group. The best known effect is that adipocyte-derived leptin acts on the hypothalamus to centrally suppress feeding and fatty acid metabolism (Baranova, 2008). Leptin and insulin compete for the same signaling molecules, in particular, JAK2/STAT-3 and phosphoinositide 3-kinase (PI3K; Baranova, 2008). As a result, an increase in leptin reduces insulin efficiency (Benomar *et al.*, 2005). Overfeeding studies consistently showed an increase in serum leptin concentrations following overfeeding (Joosen *et al.*, 2006; Brons *et al.*, 2009).

Like leptin, adiponectin is synthesized exclusively in adipocytes (Baranova, 2008). In patients with type 2 diabetes compared with subjects without diabetes, low adiponectin concentrations correlate strongly with insulin resistance (Weyer *et al.*, 2001). Adiponectin has been shown to increase insulin sensitivity in rodents by administering recombinant adiponectin to insulin-resistant rodents (Yamauchi *et al.*, 2001). Our data shows a slight down-regulation of adiponectin at the mRNA level in both groups but an increase at the protein concentration level, more so in the SUR group. An increase in circulating adiponectin seems counter-intuitive considering our participants gained weight as a result of the intervention. Ukkola *et al.* (2008) showed that serum adiponectin significantly decreased in response to 100 days of overfeeding. A

more comparable study is the one conducted by Brons *et al.* (2009) where subjects consumed a high-fat, high calorie diet for five days. They noticed an increase in fasting levels of plasma adiponectin in response to this diet. Adiponectin increases both hepatic and peripheral insulin sensitivity (Fasshauer & Paschke, 2003). Brons *et al.* (2009) hypothesised that the increase in adiponectin may explain the lack of the otherwise expected development of overt peripheral insulin resistance. This increase of circulating adiponectin points towards an acute response to the overfeeding perhaps in an attempt to improve insulin sensitivity.

Our post-intervention calculations show that carbohydrate and fat contributed approximately 20 % and 50 % towards the overall energy surplus, respectively. There was a non-significant tendency for positive carbohydrate balance (i.e., intake – oxidation – conversion to lipid) to be greater in the SUR than SUR+EX group ($1092 \pm 797 \text{ g}\cdot\text{week}^{-1}$ and $708 \pm 809 \text{ g}\cdot\text{week}^{-1}$ in SUR and SUR+EX groups, respectively; $P=0.24$). The current experiment was designed to induce a matched energy surplus with or without exercise and not to account for the route of disposal for each macronutrient. Inevitably, the SUR+EX oxidised more carbohydrate as a result of daily vigorous-intensity exercise where carbohydrate was the primary substrate but, because the overfeeding was based on their usual diet, the surplus for individual macronutrients was not matched. It is possible that the tendency for approximately $50 \text{ g}\cdot\text{d}^{-1}$ greater carbohydrate balance between groups could explain differences in insulin sensitivity at follow-up. However, research shows that exercise enhances insulin action even when expended energy and carbohydrate are replaced (Stephens *et al.*, 2007). Based on our observations, we cannot know the fate of the surplus carbohydrate. Some possibilities for disposal of surplus carbohydrate not stored as glycogen include: excretion (although this is likely to be minor; Acheson *et al.*, 1988), adaptive thermogenesis (e.g., fidgeting), and an under-estimation of DNL. Recent work by Hodson *et al.* (2013) indicates that DNL takes place after meals within the adipose tissue of healthy humans whereas we have based our calculations on only fasting measures. Furthermore, we should point out that our calculations do not take into account protein and alcohol metabolism as these would also have been oxidised (Appendix 4). We can therefore assume that we are under-

estimating the amount of excess macronutrients that have been converted into fat via DNL. Future studies replacing individual macronutrients and more carefully accounting for their fate would help understand the importance of each macronutrient in regulating insulin sensitivity and adipose tissue metabolism.

Profound short-term macronutrient imbalances also have the capacity to confound prediction of energy imbalances based on observed changes in mass. In the present study, the SUR group gained 2.7 kg and the SUR+EX group gained 1.6 kg. This may be related to differences in glycogen storage. Glycogen is stored alongside water but the ratio is unclear and is likely to vary under different conditions (Appendix 5; Olsson & Saltin, 1970; Sherman *et al.*, 1982; Piehl Aulin *et al.*, 2000). Interestingly, a study by Horton *et al.* (1995) showed that 7 days of CHO overfeeding led to a ~30 % greater weight gain than matched fat overfeeding, which is very similar to the greater weight gain observed in the SUR group in the present study. Thus, in the short-term, a given energy or nutrient imbalance will not necessarily translate into a proportional change in mass.

In summary, our study shows that short-term overfeeding combined with reduced physical activity induces a state of insulin resistance, hyperinsulinaemia and altered expression of several key genes within adipose tissue. The addition of daily vigorous-intensity exercise mostly prevented these changes independent of any net effect on energy imbalance. Whether this is facilitated by regular glycogen turnover or some other consequence of muscle contraction *per se* remains to be explored. These results demonstrate that exercise has a profound effect on physiological function even in the face of a considerable energy surplus.

CHAPTER 5

Caloric restriction combined with moderate or vigorous-intensity exercise provide similar positive changes in metabolic control when energy expenditure and caloric deficit are matched.

5.1 Introduction

In Chapter 4, we demonstrated that a daily bout of vigorous-intensity exercise prevented most of the metabolic changes induced by seven days of overfeeding and reduced physical activity independent of any net effect on energy imbalance. Although we have shown that vigorous-intensity exercise can offer substantial benefits in the context of an energy surplus, it is still unclear whether exercise-induced changes in the context of an energy deficit are specific to the characteristics of the exercise (such as duration and intensity). Whether exercise training programmes that differ in intensity and duration also differ in their ability to improve metabolic health remains uncertain.

Whilst some studies suggest that vigorous high-intensity exercise can further improve glucose control and insulin action compared to moderate-intensity exercise (Seals *et al.*, 1984; Kang *et al.*, 1996; Swain & Franklin, 2006), others have shown that moderate-intensity exercise can provide the same benefits (Oshida *et al.*, 1989; Braun *et al.*, 1995; Houmard *et al.*, 2004; O'Donovan *et al.*, 2005). To complicate matters further, there is much debate about whether the effect of exercise on metabolic control is independent of changes in energy balance. In order to determine whether vigorous-intensity exercise provides further health benefits than moderate-intensity exercise, it is crucial to control energy status during the intervention (Braun & Brooks, 2008). It is also important to prioritise health outcomes when investigating the optimum intensity, duration and frequency of a type of exercise as conclusions may vary depending on the health outcome in question (Haskell, 2001).

Houmard *et al.* (2004) showed that total exercise duration was the key determinant in improving insulin action in the Studies of a Targeted Risk Reduction Interventions through Defined Exercise (STRRIDE) study. In

STRIDE, sedentary, overweight/obese participants (n=154) were randomly assigned to either a control or an exercise group for six months. The exercise arm was further split into three different categories: 1) low-volume/moderate-intensity group (~12 miles walking/week at 40-55 % $\dot{V}O_{2\text{ peak}}$) 2) low-volume/high-intensity group (~12 miles jogging/week at 65-80 % $\dot{V}O_{2\text{ peak}}$) 3) high-volume/high-intensity group (~20 miles jogging/week at 65-80 % $\dot{V}O_{2\text{ peak}}$). Insulin sensitivity was shown to improve in all three groups but the low-volume/moderate-intensity and high-volume/high-intensity groups elicited greater improvements in insulin sensitivity than the low-volume/high-intensity group. The authors concluded that total exercise duration (regardless of exercise intensity) was the key factor in improving insulin sensitivity. Importantly, energy balance was not controlled during the intervention as there was no mention of any dietary assessment. O'Donovan *et al.* (2005) investigated the effect of exercise intensity on insulin resistance by comparing moderate and high-intensity training of equal energy cost in sixty-four previously sedentary men who were randomly allocated to either: 1) a non-exercise group 2) a moderate-intensity group 3) a high-intensity group. Participants were instructed to maintain their normal dietary habits. They concluded that moderate-intensity exercise is as effective as high-intensity exercise when 400 kcal are expended per session.

There is a strong scientific rationale for combining hypocaloric diets with regular physical aerobic exercise as the most effective treatment of obesity (Hsueh & Buchanan, 1994; Brochu *et al.*, 2000; Poirier & Despres, 2001). The combination of caloric restriction alongside increased physical activity is more ecologically valid than studies looking at diet or physical activity alone. It is unclear whether variation in exercise intensity will lead to different metabolic responses. A study by Nicklas *et al.* (2009) investigated whether varying exercise intensity concurrent with caloric restriction affected abdominal fat loss and risk factors of cardiovascular disease. In a randomised controlled trial of equal energy deficit, 112 overweight and obese postmenopausal women were assigned to one of three groups for the study duration of 20 weeks: 1) caloric restriction alone 2) caloric restriction plus moderate-intensity exercise 3) caloric restriction plus vigorous-intensity exercise. The average weight loss for all

participants was 12 ± 5 kg, they found that there was no difference in abdominal fat loss or improvements of metabolic markers across treatment groups. Roberts *et al.* (2006) also combined short-term diet manipulation with daily vigorous aerobic exercise (45-60 minutes at 70-85 % $\dot{V}O_{2\max}$) in a 3-week intervention in middle-aged obese men ($n=31$). Participants were placed on a high-fibre, low-fat diet where food was provided *ad libitum*. After 3 weeks, significant reductions ($P<0.05$) were achieved for all serum lipids, fasting glucose and insulin as well as C-reactive protein. It is uncertain whether these improvements were the result of the increased physical activity or the weight loss/negative energy balance. These findings highlight the importance of carefully controlling energy balance when investigating whether exercise intensity has an impact on metabolic outcomes and inflammatory markers.

The purpose of the present study was therefore to investigate whether caloric restriction alongside vigorous-intensity exercise would further improve metabolic control in inactive and overweight/obese men and postmenopausal women over the course of a three-week intervention relative to moderate-intensity exercise. We hypothesised that caloric restriction alongside vigorous-intensity exercise would further improve metabolic markers and the expression of key genes within adipose tissue (a major site for energy storage) during a period of negative energy balance compared to moderate-intensity exercise when caloric restriction and exercise energy expenditure are matched.

5.2 Methods

5.2.1 Overview of experimental design

The purpose of this study was to examine whether caloric restriction combined with moderate or vigorous-intensity exercise would further improve metabolic control and markers of inflammation in inactive and overweight/obese men and postmenopausal women over the course of a three-week intervention. The study duration was based on a similar protocol used by Roberts *et al.* (2006) which reduced markers of inflammation in obese middle-aged men following a three-week intervention. A randomised parallel group design was used for this study, with participants randomly allocated by a third party to experience a fixed

energy deficit of $29330 \text{ kJ}\cdot\text{week}^{-1}$, induced by caloric restriction and increased physical activity via either moderate (MOD) or vigorous (VIG) physical exercise. Volunteers in both groups were asked to reduce their caloric intake by under-consuming their habitual diet (Chapter 2.9) in order to create a caloric deficit of $20950 \text{ kJ}\cdot\text{week}^{-1}$. In addition, participants in the MOD group increased their physical activity by walking on a treadmill five times per week at 50 % of their maximum oxygen uptake while participants in the VIG group increased their physical activity by walking on a treadmill five times per week at 70 % of their maximum oxygen uptake. Importantly, both groups expended $8380 \text{ kJ}\cdot\text{week}^{-1}$ (1676 kJ per session for both groups) above rest through increased physical activity.

Changes in insulin sensitivity and glycaemic control reflected by basal values and incremental Area Under the Curve (iAUC) measured at baseline and follow up were the primary outcome measures. Other blood measures related to metabolic control, inflammation and changes within adipose tissue were secondary outcome measures.

5.2.2 Participants

Thirty-eight inactive and overweight ($\text{BMI} > 25 \text{ kg}\cdot\text{m}^{-2}$) men ($n=24$) and postmenopausal women ($n=14$) completed the present study. Participants were 45-64 years old with a mean age of 52 ± 5 years (mean \pm SD) and had been weight stable ($\pm 3 \text{ kg}$) for at least 6 months. Women were required to have been postmenopausal in order to take part in the study (no menstruation for at least a year; Witteman *et al.*, 1989). To be eligible, participants were required to report no participation in regular structured exercise and that they did not do >30 minutes of moderate-intensity exercise, accumulated in 10 minutes bouts, on most days of the week. Habitual physical activity was assessed via a physical activity questionnaire (Appendix 2). Participants completed a health questionnaire (Appendix 2) and Physical Activity Readiness Questionnaire (PAR-Q; Appendix 3) to determine eligibility and provided written and verbal consent consistent with the requirements of the Bath NHS Research Ethics Committee, who approved this study (REC reference number: 08/H0101/194). Specifically, individuals who smoked, suffered from a condition known to

interact with the study measures or took regular medication that may have interfered with the results were excluded from the study. All participants confirmed they adhered to the exercise training by filling out an exercise log and attending a weekly supervised exercise session. They also confirmed to have only consumed the prescribed foods over the course of the intervention. The anthropometric and physiological characteristics of those included are presented in Table 5.1.

Table 5.1: Participant details. Mean \pm SD

Characteristic	MOD group (n=20)	VIG group (n=18)
Age (y)	53 \pm 6	50 \pm 4
Height (m)	1.71 \pm 0.10	1.71 \pm 0.12
Mass (kg)	90.0 \pm 13.6	89.5 \pm 16.6
BMI (kg·m ⁻²)	30.7 \pm 2.4	30.3 \pm 2.7
Fat Mass Index (kg·m ⁻²)	11.2 \pm 2.1	10.7 \pm 2.2
$\dot{V}O_2$ max (ml·kg ⁻¹ ·min ⁻¹)	31.7 \pm 4.2	31.3 \pm 4.9
$\dot{V}O_2$ max (l·min ⁻¹)	2.9 \pm 0.6	2.9 \pm 0.8
PAL (TEE/REE)	1.57 \pm 0.17	1.59 \pm 0.17

5.2.3 Design Summary

Participants were randomly-allocated by a third party to one of two groups for the study period of three weeks using block randomisation:

- 1) Caloric restriction with vigorous-intensity exercise (VIG) n=20
- 2) Caloric restriction with moderate-intensity exercise (MOD) n=18

This resulted in 13 males and 7 females in the MOD group and 11 males and 7 females in the VIG group.

The experimental design is summarised below (Figure 5.1)

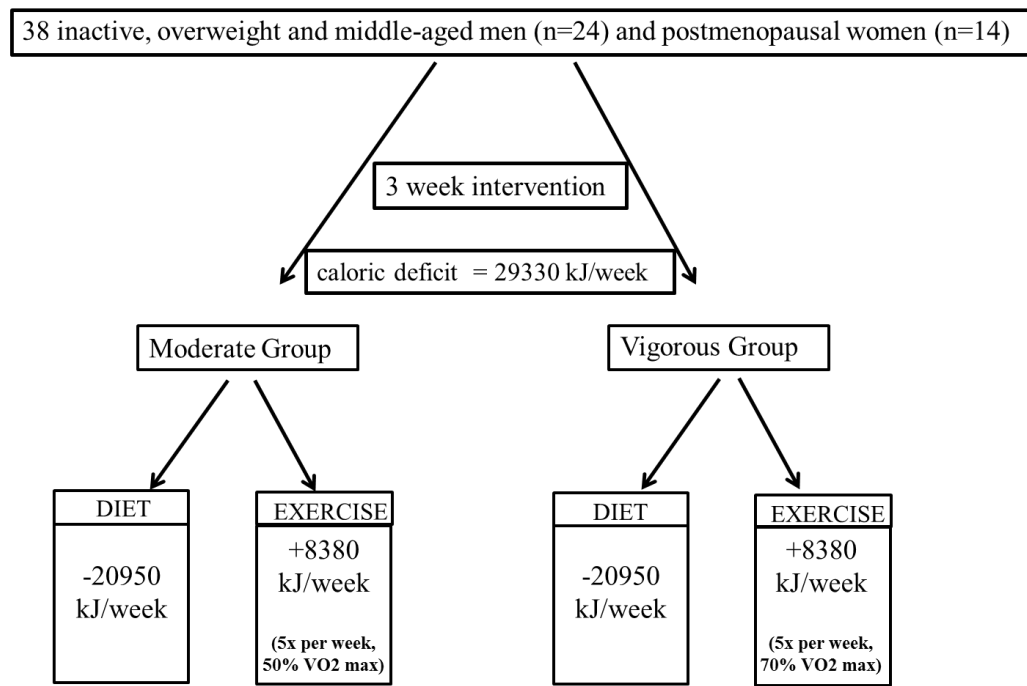


Figure 5.1: Schematic representation of the energy deficit induced by the caloric restriction and increased physical activity model.

In the month prior to the three-week intervention, participants recorded their food and fluid intake for one week as described in Chapter 2.9, which was used to calculate the energy intake required to cause an energy deficit of 20950 kJ·week⁻¹ from caloric restriction alone. This was achieved by subtracting 20950 kJ from their recorded total weekly energy expenditure (assessed by the Actiheart during the diet recall week). The dietary caloric restriction was based on participants' energy expenditure as overweight and obese individuals have been shown to under-report food intake (Macdiarmid & Blundell, 1998). Based on this, participants recorded diets were scaled in order to induce the desired energy deficit during the intervention. This was achieved by multiplying the weight of all individual foods by the desired energy intake over the recorded week energy input. Participants repeated this one-week diet three times during the intervention. Participants underwent a maximal oxygen uptake test in order to determine the correct speed and gradient required for them to achieve either 50 % $\dot{V}O_2$ max for the MOD group or 70 % $\dot{V}O_2$ max for the VIG group (Chapter 2.6). A corresponding Heart Rate (HR) at this gradient and speed was estimated from the relationship between HR and % $\dot{V}O_2$ max. Energy expenditure at the desired intensity was utilised to calculate a set distance (km)

required to expend 1676 kJ above resting energy expenditure. In order to account for post-exercise oxygen consumption, measured oxygen consumption was adjusted by an extra 5.4 % for the MOD group and by 6.4 % for the VIG group (Gore & Withers, 1990). Participants were asked to complete this distance at predicted HR below 50 % $\dot{V}O_{2\max}$ for the MOD group or at least 70 % $\dot{V}O_{2\max}$ for the VIG group. Exercise took place in the Sports Training Village fitness suite at the University of Bath. In addition, one supervised exercise session per week was performed in the Exercise Physiology Laboratory. Assuming a linear relationship between speed and energy expenditure when walking at a set gradient (Franklin, 2000), participants were then able to adjust the speed in order to achieve the target heart rate and intensity.

Participants abstained from tea/coffee or alcohol the day before each trial. During the days leading up to the trial, volunteers followed their normal lifestyle and consumed their habitual diet. The final exercise bout prescribed was performed 48 hours before follow-up measurements were collected.

5.2.4 Trial Days

Following an overnight fast (≥ 10 h), participants arrived in the laboratory at 0700 ± 0.5 h. Body mass was measured to the nearest 0.1 kg using electronic scales (Tanita Corporation, Japan). All participants wore the same light clothing at baseline and follow-up for body mass measurement. Anthropometric measurements were made in triplicate using a metallic tape measure (Lufkin, US) before lean and fat mass were measured using Dual-Energy X-ray Absorptiometry (DEXA) (Discovery, Hologic, Bedford, UK) (Chapter 2.2). Following the application of a topical local anaesthetic (1.5 ml Ametop gel, Smith & Nephew, Hull, England) an 18-gauge 1.3x45 mm cannula (BD Venflon Pro) was inserted into an antecubital vein and a baseline blood sample collected. An adipose tissue sample was then collected (Chapter 2.10) before an OGTT was performed according to the procedure described in Chapter 2.4. Blood pressure was measured in triplicate at the end of each trial (Chapter 2.3). The same protocol was used at follow-up.

5.2.5 Analytical methods

Blood was analysed for WBC counts, serum CRP, IL-6, Adiponectin, Leptin, ALT, Insulin, TAG, Cholesterol, HDL-Cholesterol, NEFA and plasma glucose. Adipose tissue was analysed for mRNA (Chapter 2.11, 2.12).

5.2.6 Statistical analysis

To simplify data analysis and facilitate a more meaningful interpretation of an otherwise complex factorial research design (Hopkins *et al.*, 2009 & Matthews *et al.*, 1990), serial measurements of glucose and insulin at baseline and follow-up were converted into simple summary statistics to illustrate the net response of each parameter (i.e. within-subject peak concentrations, time to peak and incremental area under curve; Wolever & Jenkins, 1986). Pre-planned contrasts were conducted in relation to the absolute group differences both at final follow-up and the relative change from baseline. The precise time-course of responses within and between trials were analysed using factorial 2- and 3-way mixed-model analysis of variance (group \times day & group \times day \times time, respectively) irrespective of minor deviations from a normal distribution (Maxwell & Delaney, 1990) but with the Greenhouse-Geisser correction applied to intra-individual contrasts for $\epsilon < 0.75$ and the Huynh-Feldt correction adopted for less severe asphericity (Atkinson, 2002). Where significant interactions were observed, multiple t-tests were applied to determine the location of variance both between treatments at each time point and between time points within each treatment relative to baseline, with both methods subject to a Holm-Bonferroni correction (Atkinson, 2002). For all the above statistical approaches, statistical significance was set at an alpha level of $P \leq 0.05$.

Data are presented in text as means and standard deviations (SDs). The confidence intervals displayed in tables are standard 95 % confidence intervals (95 % CI) to better illustrate group differences whereas the variance bars on figures are normalised confidence intervals (nCI) that have been corrected to remove inter-individual variation (Masson, 2003). For reference, the magnitude of these CIs illustrate the change at each time point relative to baseline such that, in general, plotted means whose CIs do not overlap by more than one-half of one side of an interval are likely to be deemed statistically different according

to conventional significance testing (Masson, 2003). A main effect of day denotes an effect of energy surplus (†: Day 1 vs. Day 21 both groups), whereas a day \times group interaction means there is a mediating effect of exercise (*). Statistical analysis for the gene expression data was carried out on the ΔC_t values. The Pearson rank correlation was employed to determine the strength of relationships between parameters. For CRP and IL-6, the mean and standard deviation of the change (Δ) were calculated using all data points. Any change score that was over 3 SD away from the mean were excluded from analysis as this would likely have been due to an acute inflammatory response. This is mentioned in the Figure legend when this rule has been applied.

5.3 Results

5.3.1 Anthropometric and physiological measures

Anthropometric and physiological measures pre and post-intervention are summarised in Table 5.2. Body mass, waist & hip circumference, fat mass (DEXA), abdominal fat (DEXA), systolic and diastolic blood pressure significantly decreased post-intervention in both groups; there was a main effect of day ($P < 0.001$) as shown in Table 5.2. Lean mass (DEXA) was not affected by the intervention in either group.

Table 5.2: Anthropometric and physiological characteristics measured before and after 3 weeks of caloric restriction with moderate-intensity physical exercise (MOD; n=20) or vigorous-intensity physical exercise (VIG; n=18) (n.b. waist and hip measurements n=19 in MOD group; blood pressure measurements n=17 in VIG group). Mean \pm SD. Change scores shown with 95% confidence intervals. † main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F > 23.2$, $P < 0.001$).

	MOD			VIG		
	Baseline	Follow-up	Δ (95% CI)	Baseline	Follow-up	Δ (95% CI)
Body Mass (kg)	90.0 \pm 13.6	87.6 \pm 13.3	-2.4 (1.7 to 3.1) †	89.5 \pm 16.6	87.1 \pm 16.6	-2.4 (1.9 to 2.9) †
Waist Circumference (cm)	104.7 \pm 6.8	102.9 \pm 6.5	-1.8 (0.9 to 2.7) †	101.0 \pm 10.4	98.8 \pm 10.5	-2.2 (0.9 to 3.4) †
Hip Circumference (cm)	111.0 \pm 5.9	109.3 \pm 5.0	-1.6 (0.7 to 2.8) †	110.6 \pm 6.2	108.2 \pm 5.0	-2.4 (1.4 to 3.5) †
Fat Mass (kg; DEXA)	32.3 \pm 5.6	30.5 \pm 5.0	-1.8 (0.9 to 2.8) †	30.9 \pm 5.4	29.0 \pm 5.1	-1.9 (1.2 to 2.6) †
Lean Mass (kg; DEXA)	54.2 \pm 11.4	53.5 \pm 11.0	-0.7 (-0.4 to 1.8)	54.8 \pm 14.7	54.5 \pm 14.9	-0.3 (-1.1 to 0.5)
Abdominal Fat (kg; DEXA)	4.8 \pm 1.4	4.4 \pm 1.3	-0.4 (4.2 to 5.5) †	4.4 \pm 1.1	3.9 \pm 1.0	-0.4 (3.8 to 4.9) †
Systolic BP (mmHg)	128 \pm 13	121 \pm 15	-7 (3 to 10) †	135 \pm 11	128 \pm 13	-7 (2 to 12) †
Diastolic BP (mmHg)	80 \pm 12	75 \pm 10	-5 (1 to 8) †	85 \pm 9	79 \pm 9	-6 (3 to 9) †

5.3.2 Insulin & Glucose responses to OGTT

Serum Insulin

The serum insulin concentrations in response to the OGTT were significantly reduced at follow-up for both groups as shown in Figure 5.2. There was a main effect of day ($P=0.001$). The insulinaemic response following the OGTT was significantly reduced at follow-up for both groups with a decrease in 2 h iAUC; and there was a main effect of day ($P=0.005$) as shown by Figure 5.3. There also was a main effect of day ($P=0.009$) for the serum insulin peak concentration of both groups following the 2 h OGTT as shown by Figure 5.4. The individual peak serum insulin time was unaffected in both groups.

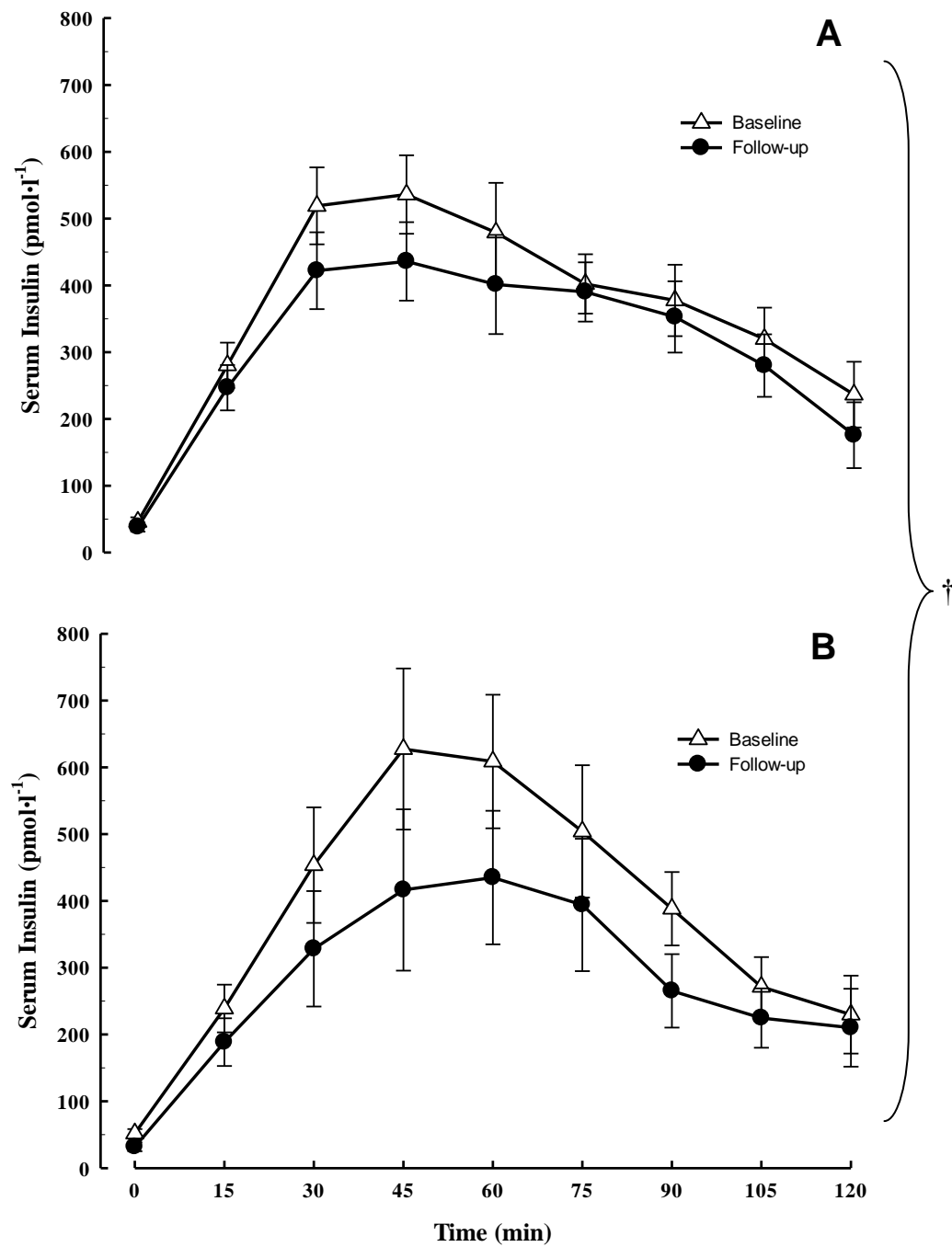


Figure 5.2: Serum insulin concentrations in response to the OGTT before and after 3 weeks of caloric restriction and increased physical activity (Panel A: MOD group, $n=15$; Panel B: VIG group, $n=14$). Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=15.1$, $P=0.001$).

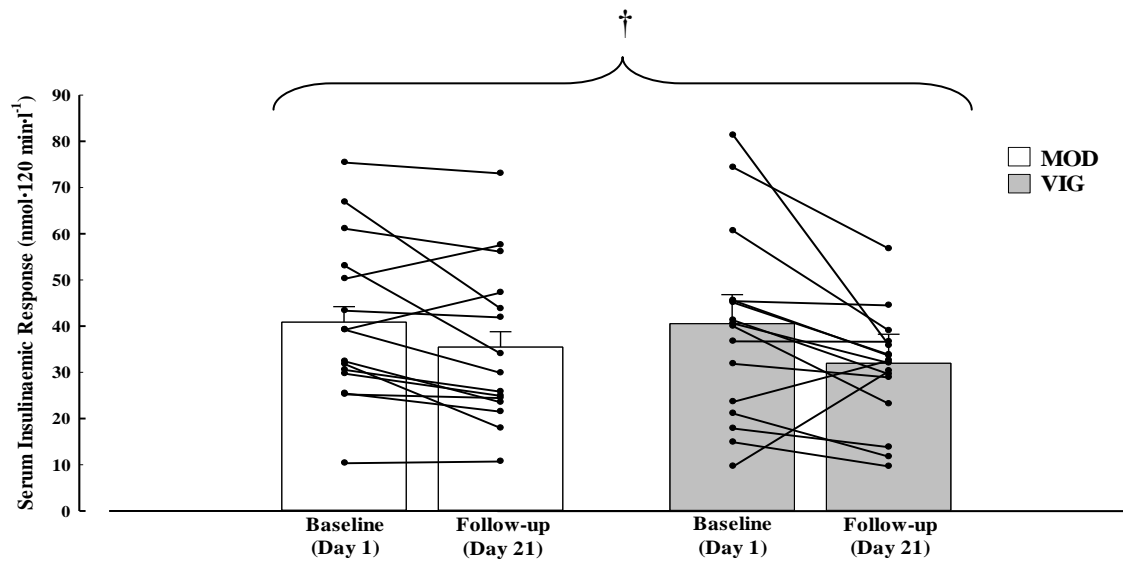


Figure 5.3: Insulinaemic responses during the 2 h OGTT for the MOD group (n=14) and the VIG group (n=15). Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; F=9.4, P=0.005).

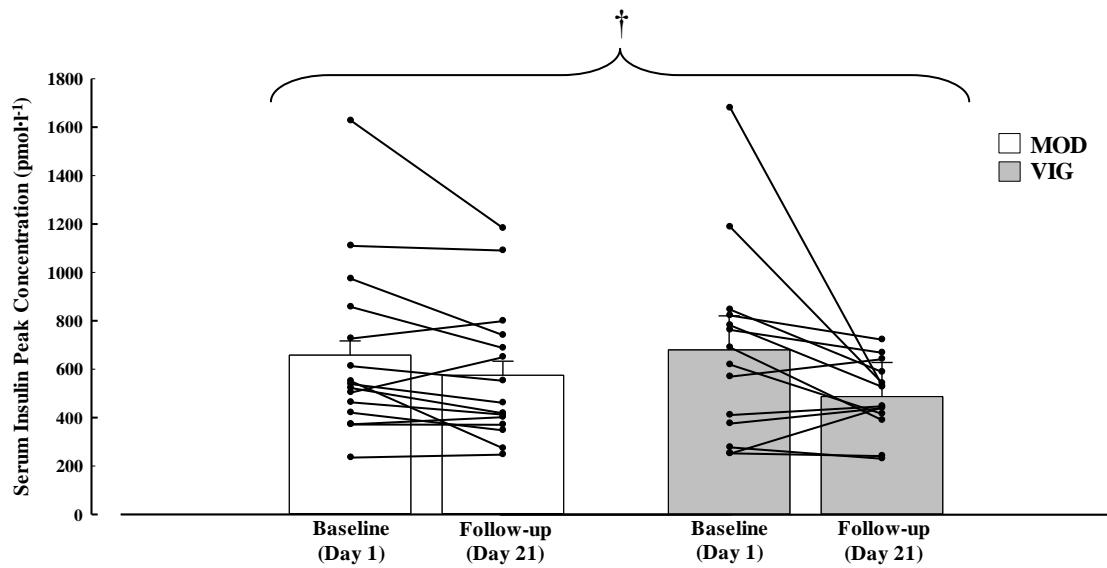


Figure 5.4: Serum insulin peak concentration during the 2 h OGTT for the MOD group (n=14) and the VIG group (n=14). Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; F=8.0, P=0.009).

Plasma Glucose

The glycaemic response following the OGTT was unaffected in both groups as shown by Figure 5.5. There was a day×group interaction ($P=0.04$) for the iAUC of the glycaemic response following the OGTT as shown by Figure 5.6. There also was a day×group interaction ($P=0.05$) for the plasma glucose peak concentration following the 2 h OGTT as shown in Figure 5.7. The individual plasma glucose peak time was unaffected in both groups.

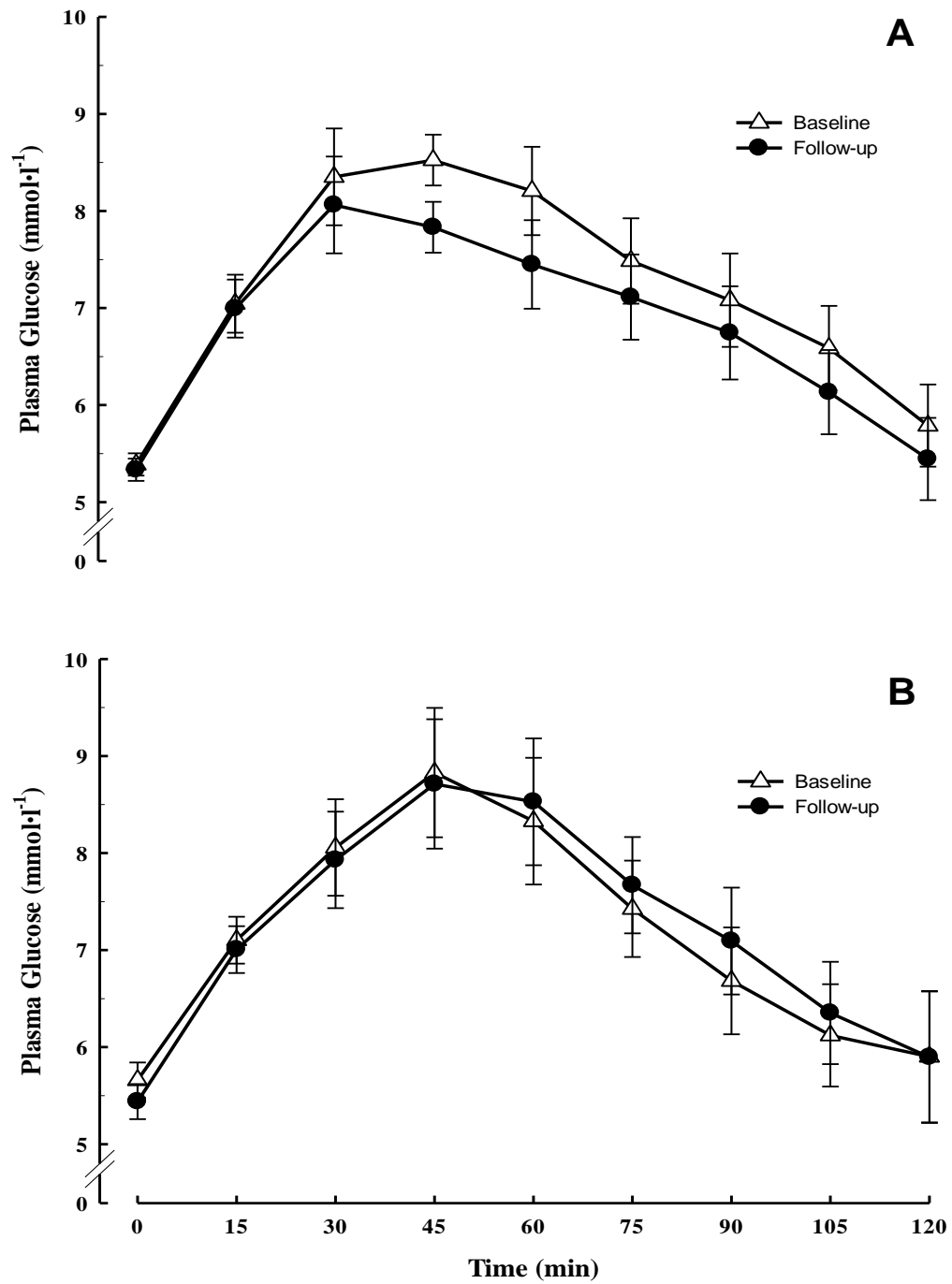


Figure 5.5: Plasma glucose concentrations in response to the OGTT before and after 3 weeks of caloric restriction and increased physical activity (Panel A: MOD group, n=16; Panel B: VIG group, n=12). Values are means \pm nCI.

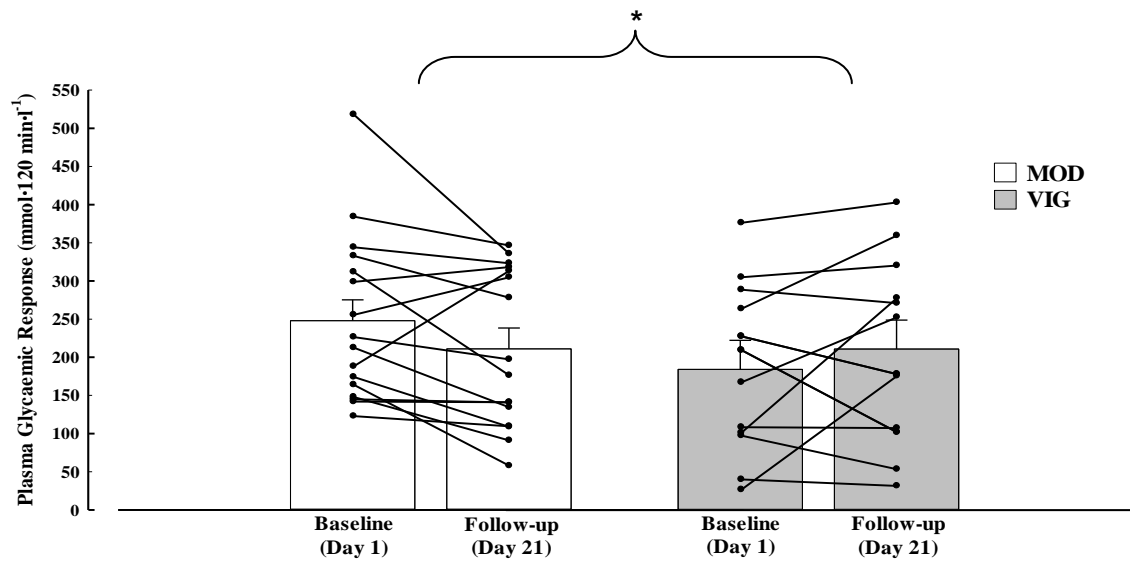


Figure 5.6: Glycaemic responses during the 2 h OGTT for the MOD group (n=16) and the VIG group (n=12). Values are means \pm nCI. * denotes a dayxgroup interaction ($F=4.6$, $P=0.04$).

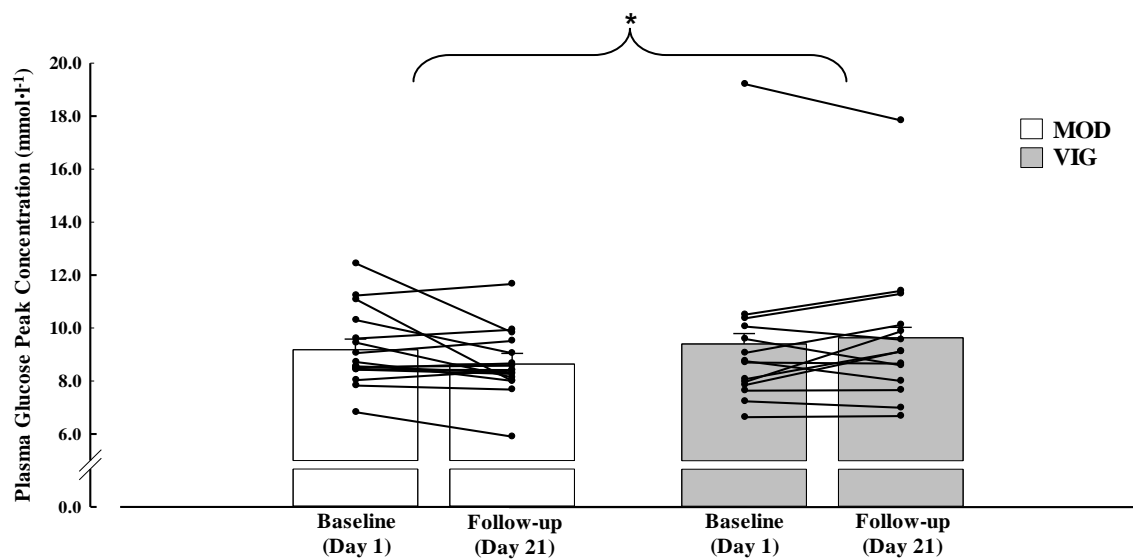


Figure 5.7: Plasma glucose peak concentration during the 2 h OGTT for the MOD group (n=16) and the VIG group (n=14). Values are means \pm nCI. * denotes a dayxgroup interaction ($F=4.1$, $P=0.05$).

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) decreased at follow-up in both groups; there was a main effect of day ($P=0.001$). The Homeostasis Model Assessment of β -cell function (HOMA- β) decreased at follow-up in both groups; there was a main effect of day ($P=0.001$). The Matsuda Index or Composite Index (ISI comp) increased at follow-up in both groups; there was a main effect of day ($P=0.007$). Data is summarised in Table 5.3.

Table 5.3: HOMA-IR (MOD, n=16; VIG, n=18), HOMA- β (MOD, n=16; VIG, n=18) and Composite Index (MOD, n=15; VIG, n=11) values at baseline and follow-up. Mean \pm SD. Change scores shown with 95% confidence intervals. † main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F>8.6$, $P<0.01$).

	MOD			VIG		
	Baseline	Follow-up	Δ (95% CI)	Baseline	Follow-up	Δ (95% CI)
HOMA-IR	1.98 \pm 1.06	1.65 \pm 0.82	-0.33 (-0.17 to 0.82) †	2.36 \pm 2.18	1.41 \pm 1.45	-0.95 (0.44 to 1.46) †
HOMA-β (%)	99 \pm 58	76 \pm 33	-22 (-4 to 48) †	84 \pm 44	52 \pm 19	-31 (13 to 50) †
Insulin Sensitivity Index (comp)	5.6 \pm 3.6	6.1 \pm 2.6	0.5 (-1.0 to 2.1) †	5.9 \pm 3.3	8.1 \pm 3.5	2.2 (1.2 to 3.3) †

5.3.3 Fasted Blood measurements

Serum adiponectin and leptin concentrations significantly decreased at follow-up in both groups; there was a main effect of day ($P=0.002$ & $P<0.001$, respectively) as shown by Figure 5.8 & 5.9. Serum cholesterol, HDL cholesterol, LDL cholesterol & TAG concentrations significantly decreased at follow-up in both groups; there was a main effect of day ($P=0.006$, $P=0.05$, $P=0.02$ & $P=0.002$, respectively) as shown by Figures 5.10, 5.11, 5.12 & 5.13, respectively. Serum NEFA concentrations were unaffected in both groups (Figure 5.14). Serum ALT activity decreased at follow-up in both groups; there was a main effect of day ($P=0.01$) as shown by Figure 5.15. Whole blood WBC & serum CRP decreased at follow-up in both groups; there was a main effect of day ($P<0.001$ & $P=0.001$, respectively) as shown by Figure 5.16 & 5.17, respectively. Serum IL-6 concentrations were unaffected in both groups (Figure 5.18).

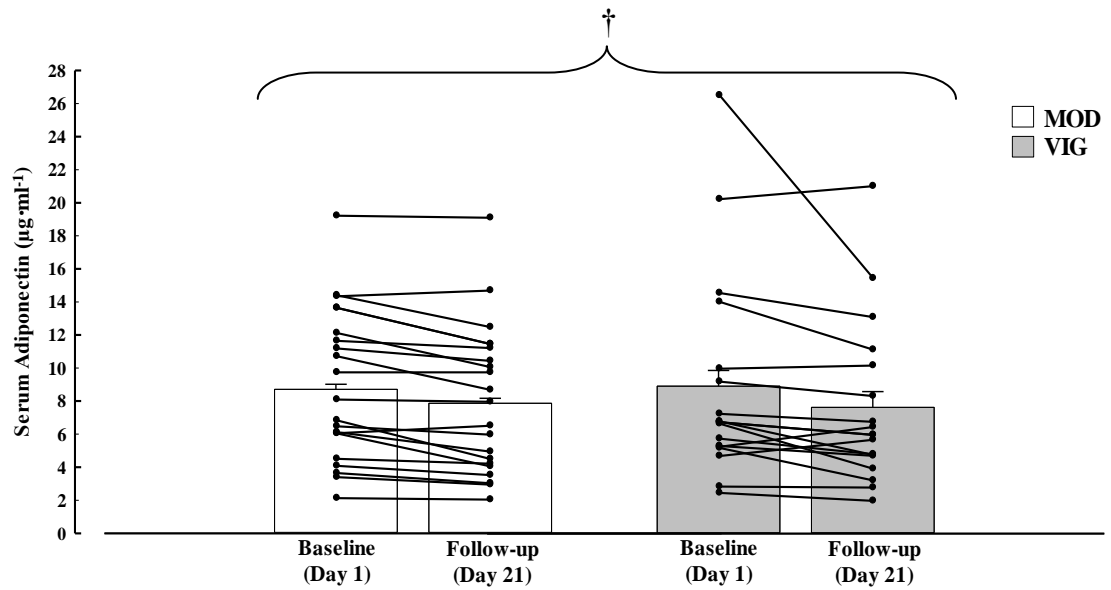


Figure 5.8: Serum Adiponectin concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=11.0$, $P=0.002$).

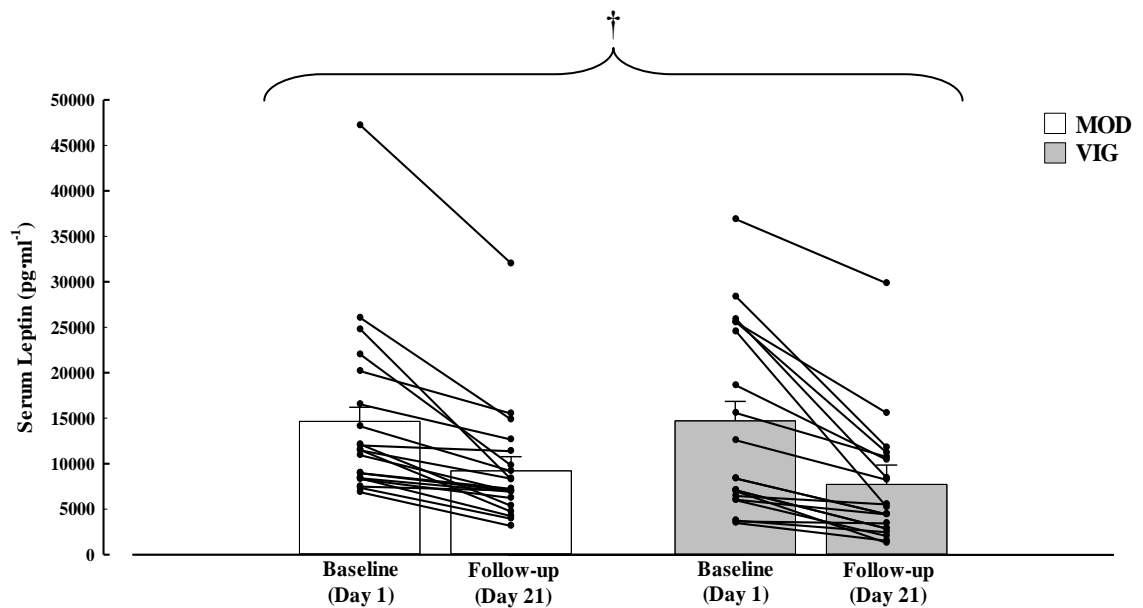


Figure 5.9: Serum Leptin concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=50.3$, $P<0.001$).

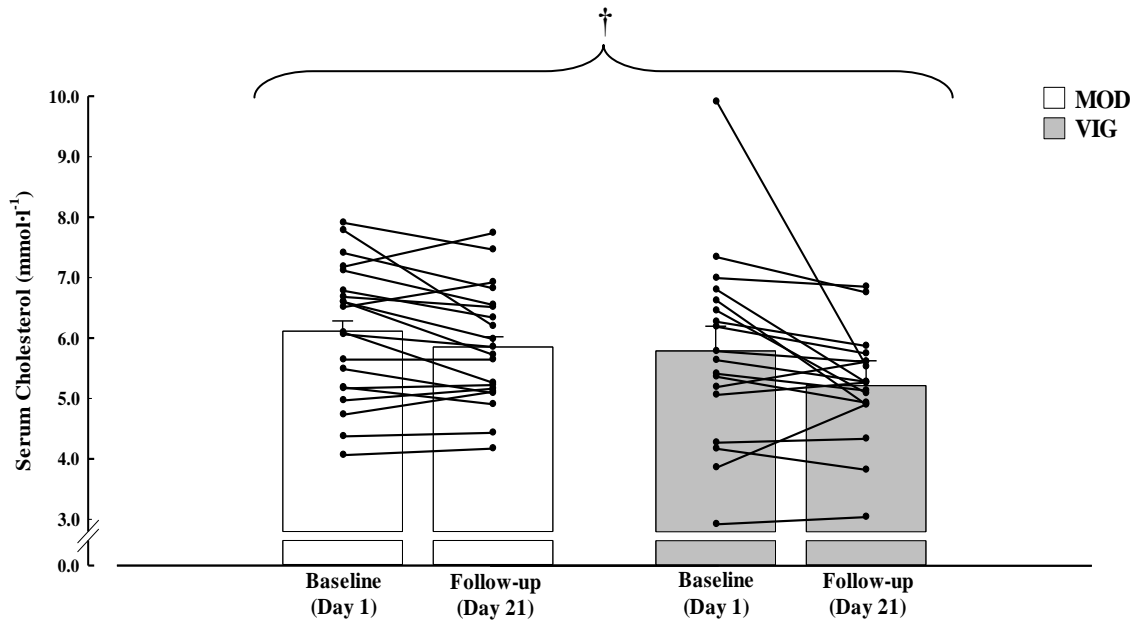


Figure 5.10: Total serum cholesterol concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=8.5$, $P=0.006$).

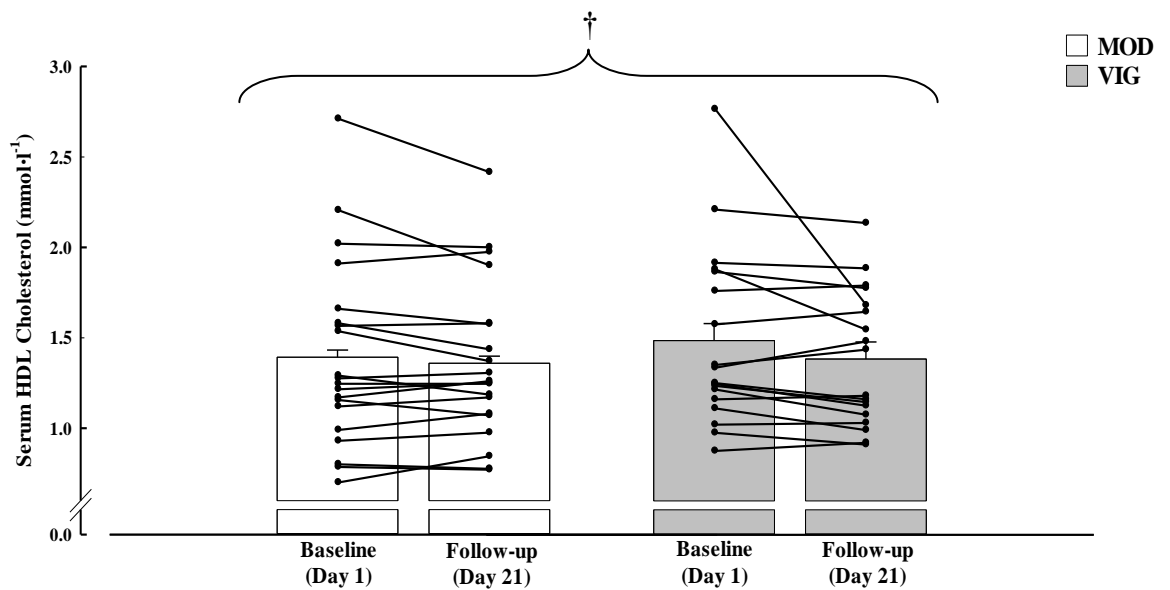


Figure 5.11: Serum HDL cholesterol concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=4.2$, $P=0.05$).

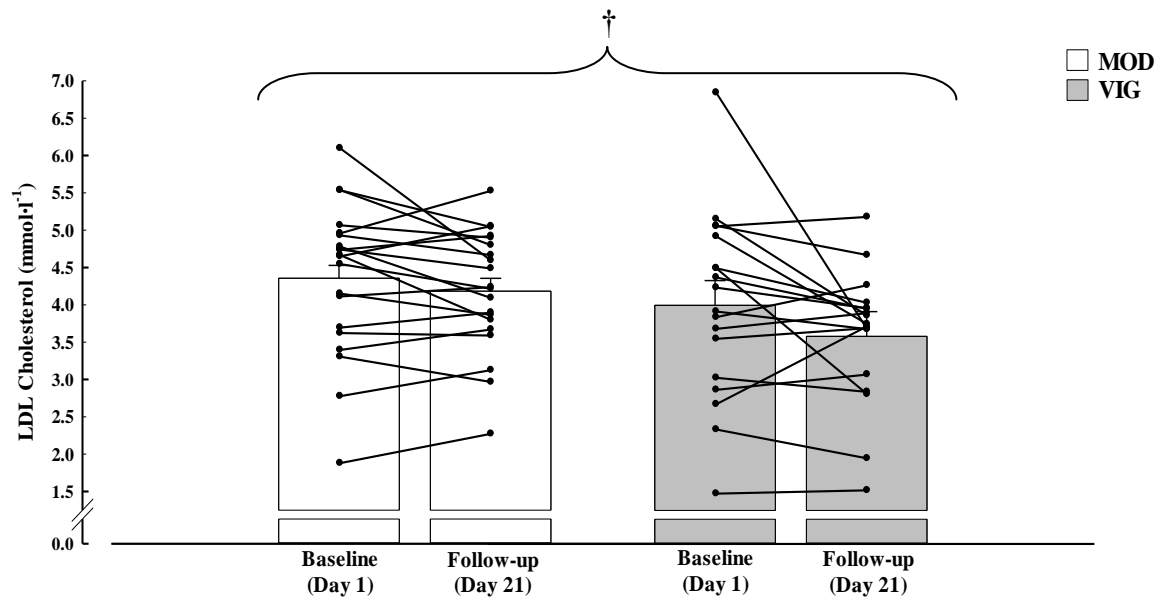


Figure 5.12: LDL cholesterol at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=5.8$, $P=0.02$).

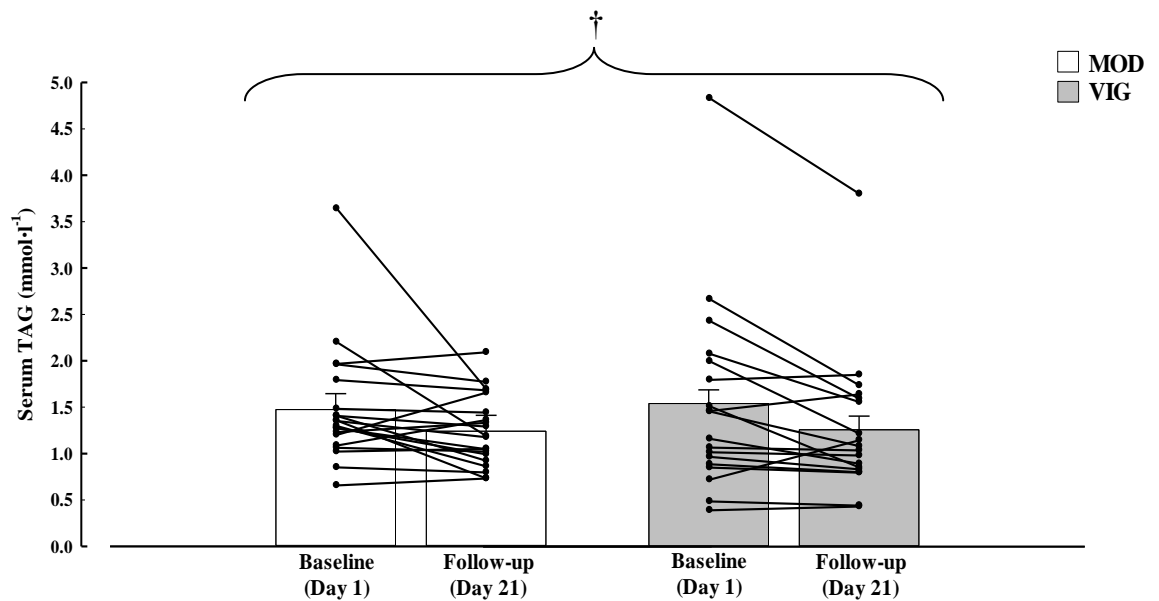


Figure 5.13: Serum triglycerides (TAG) concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=11.4$, $P=0.002$).

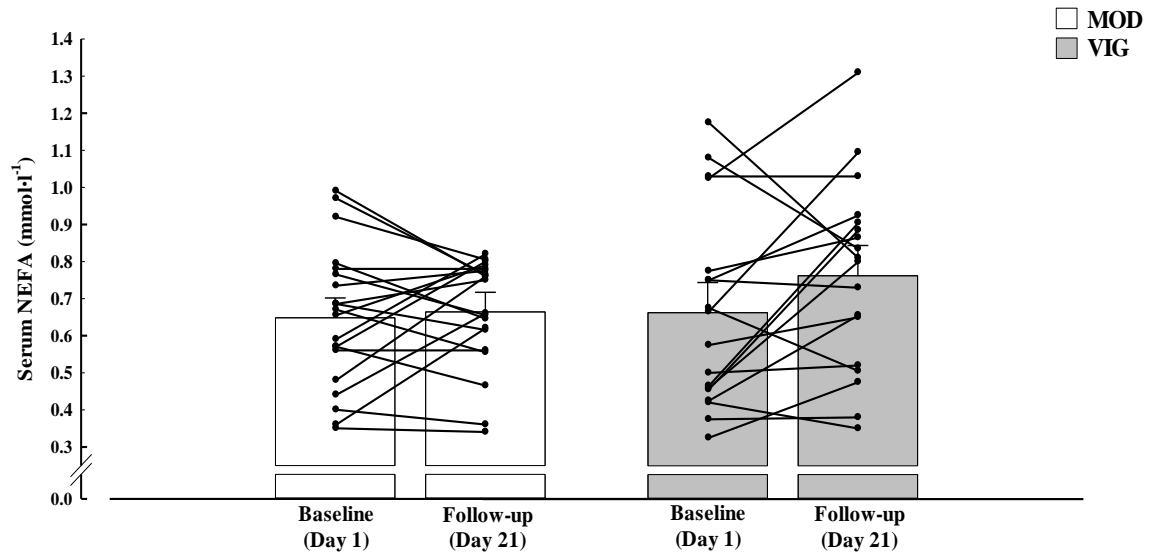


Figure 5.14: Serum NEFA concentrations at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI.

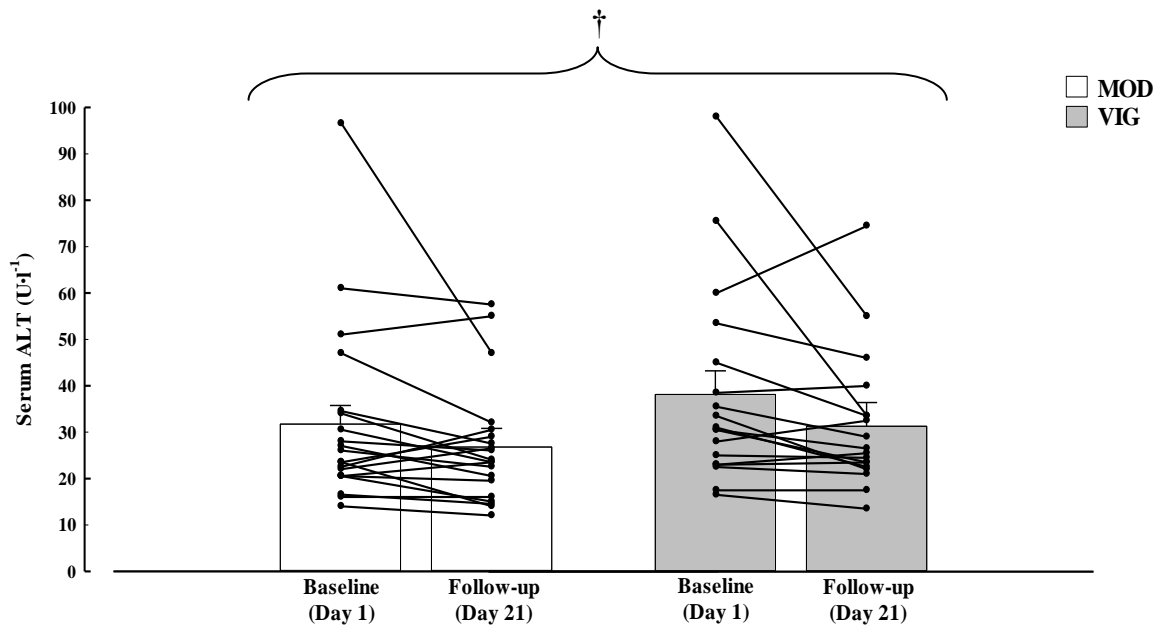


Figure 5.15: Serum ALT activity at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=7.4$, $P=0.01$).

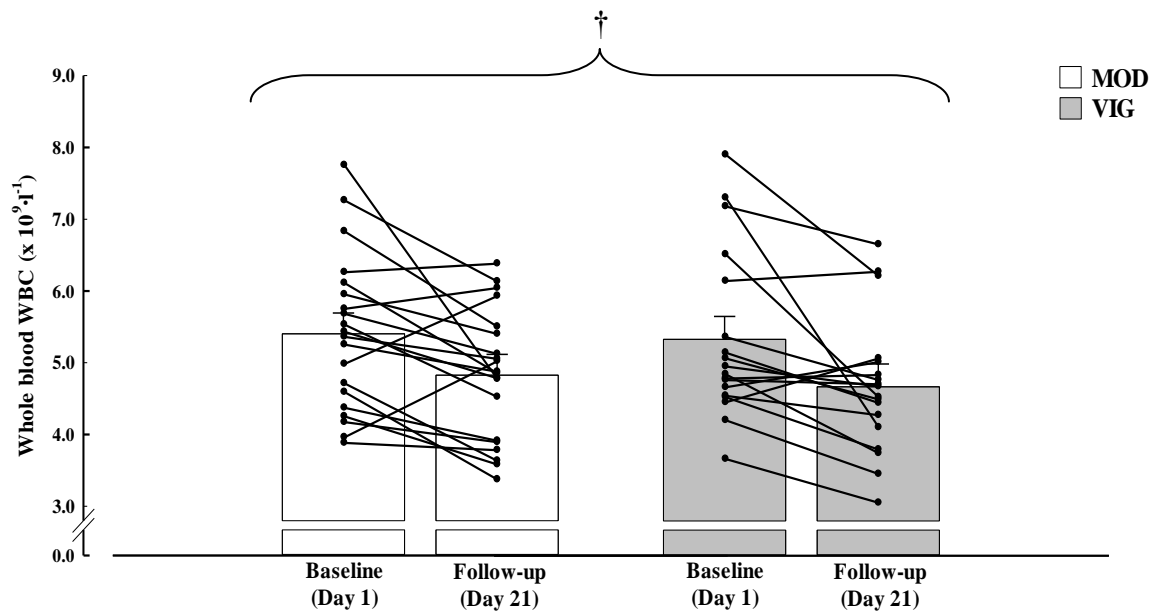


Figure 5.16: Whole blood white blood cell count at baseline and follow-up for the MOD group and the VIG group. Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=18.4$, $P<0.001$).

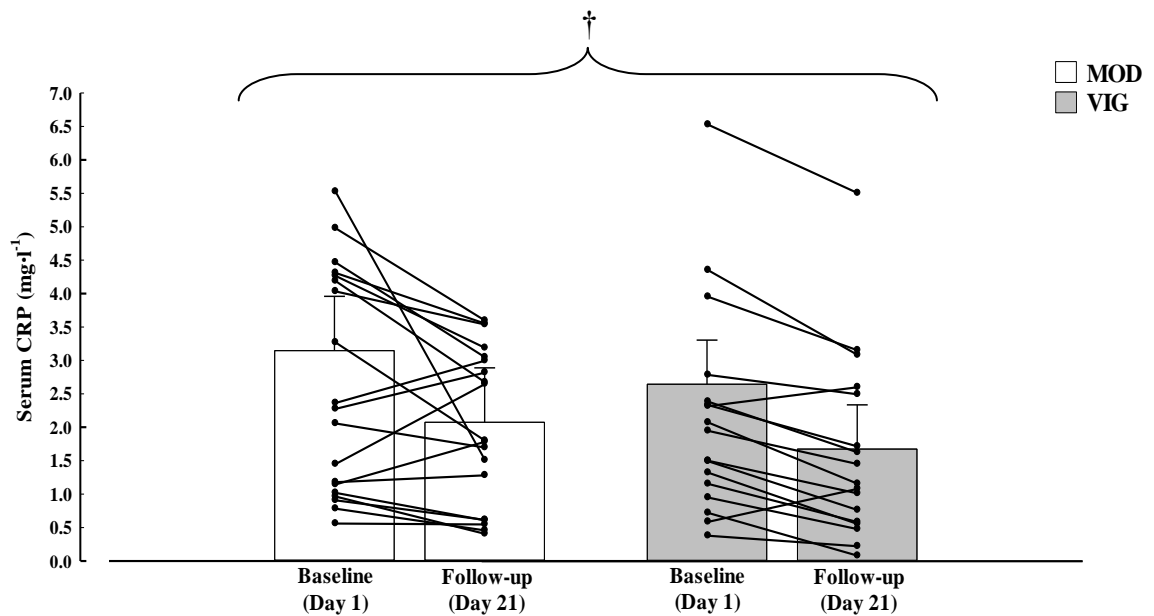


Figure 5.17: Serum CRP concentrations at baseline and follow-up for the MOD group ($n=19$) and the VIG group ($n=17$). Values are means \pm nCI. † denotes a main effect of day (i.e.: Day 1 vs. Day 21 both groups; $F=14.5$, $P=0.001$). Two participants were removed as $\Delta>3\text{SD}$.

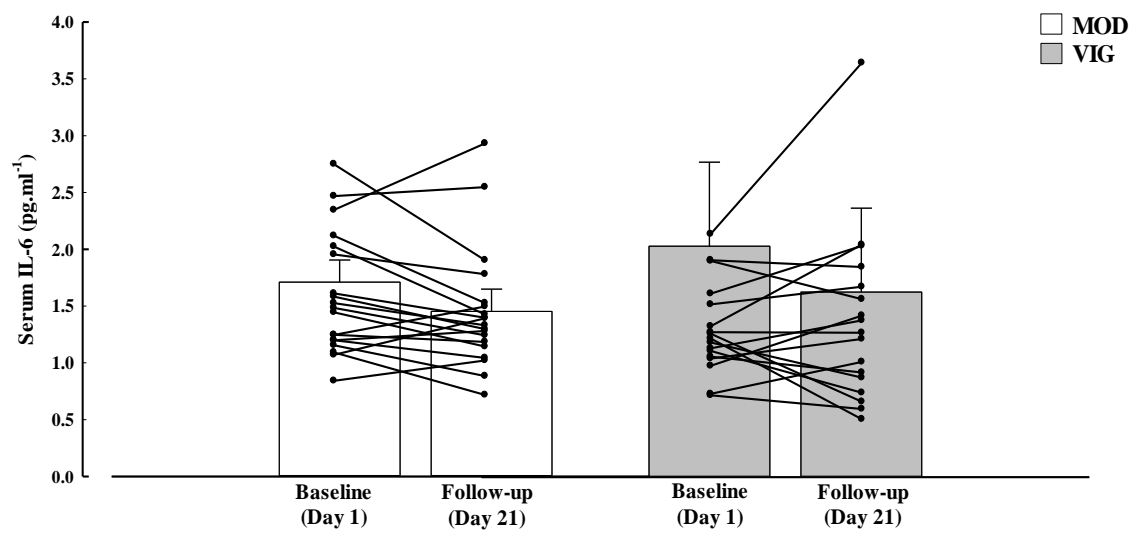


Figure 5.18: Serum IL-6 concentrations at baseline and follow-up for the MOD group (n=19) and the VIG group (n=17). Values are means \pm nCI.). Two participants were removed as $\Delta > 3SD$.

5.3.4 Adipose tissue Gene Expression

Expression of SREBP1c & FAS was significantly down-regulated in both groups; there was a main effect of day ($F>10.1$, $P<0.01$). Expression of PDK4 & HSL was significantly up-regulated in both groups; there was a main effect of day ($F>4.8$, $P\leq 0.05$). Expression of APELIN was significantly up-regulated in the MOD group ($n=5$); there was a day \times group interaction ($F=5.5$, $P=0.03$). Individuals with one or both samples outside the detectable limit ($Ct>35$) were excluded from the analysis. Two genes were expressed at very low levels; APELIN was undetectable in 29 % of samples; IL-18 was undetectable in 17 % of samples. Gene expression data is presented below (Figure 5.19). Adipose tissue biopsies were collected from a subset of participants, as a result $n=12$ in both groups.

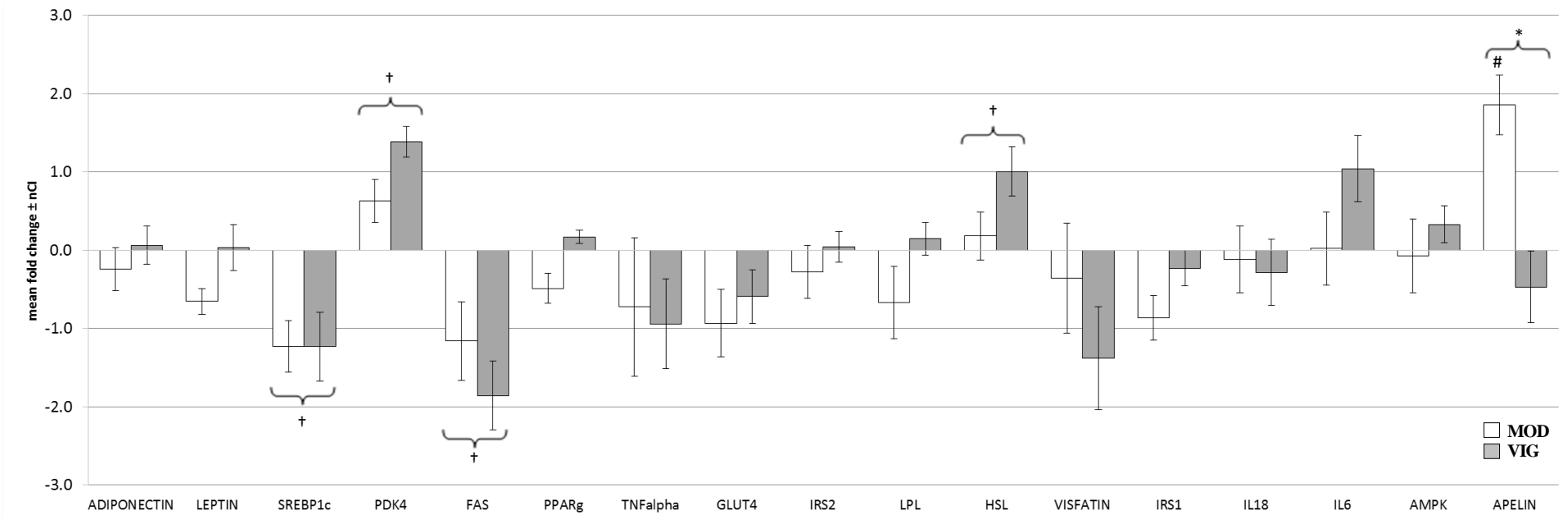


Figure 5.19: Mean fold change in the expression of several key genes measured in adipose tissue at baseline and follow-up for the MOD group (n=12) and the VIG group (n=12). Data normalised to PPIA= Peptidylprolyl isomerase A. SREBP-1c= Sterol regulatory element binding protein 1c, PDK4= Pyruvate dehydrogenase kinase isozyme 4, FAS= Fatty acid synthase (MOD, n=11), PPARγ= Peroxisome proliferator-activated receptor gamma, TNFα= Tumor necrosis factor alpha, GLUT4= Glucose transporter type 4, IRS2= Insulin receptor substrate 2, LPL= Lipoprotein lipase, HSL= Hormone-sensitive lipase. IRS1= Insulin receptor substrate 1, IL18= Interleukin-18 (MOD, n=8), IL6= Interleukin-6 (MOD, n=10; VIG, n=11), AMPK= AMP-activated kinase, APELIN (MOD, n=5). Values are means ± nCI. * $P \leq 0.05$ day x group interaction. # $P \leq 0.05$ baseline *versus* follow-up. † main effect of day (i.e.: Day 1 vs. Day 8 both groups; $P \leq 0.05$).

5.4 Discussion

The current study assessed the impact of caloric restriction combined with either moderate or vigorous-intensity exercise on metabolic control and inflammatory markers in inactive and overweight/obese men and post-menopausal women, with caloric restriction and exercise energy expenditure carefully matched between groups. We demonstrated that three weeks of caloric reduction and increased physical activity improved fasting measures of metabolic control and inflammation, responses to an OGTT and expression of several key genes within adipose tissue. These positive changes were not affected by exercise intensity.

In order to investigate whether vigorous-intensity exercise offers added benefits compared to moderate-intensity exercise in improving metabolic control and inflammatory markers in an inactive and overweight/obese population, we used a model where caloric restriction and physical exercise energy expenditure were carefully matched, as their combination has been successfully used in several other studies for similar purposes (You *et al.*, 2004; Roberts *et al.*, 2006; Nicklas *et al.*, 2009). We successfully induced a standardised negative energy balance, resulting in a weight loss of 2.4 ± 1.4 kg and 2.4 ± 1.1 kg in the MOD and VIG group, respectively. Our data suggest that after three weeks of caloric restriction and increased physical exercise, improvements in insulin sensitivity and metabolic control were evident. Interestingly, the majority of these changes were independent of exercise intensity. Short-term negative energy balance impacts metabolic control whether that is induced through caloric restriction alone (Weiss *et al.*, 2006; Fontana *et al.*, 2007; Lagerpusch *et al.*, 2011) or exercise alone (Oshida *et al.*, 1989; Kang *et al.*, 1996; Tjonna *et al.*, 2008).

A review of epidemiological data by Swain & Franklin (2006) examined a number of epidemiologic studies and clinical trials that investigated the value of moderate *versus* vigorous-intensity aerobic exercise (without caloric restriction) in improving cardiovascular health and reducing the risk of coronary heart disease. Importantly, only experiments where energy expenditure was controlled were examined. They concluded that if the total energy expenditure of exercise is held constant, exercise performed at a vigorous intensity appears

to further reduce the risk of cardiovascular disease compared to moderate-intensity exercise. In contrast, O'Donovan *et al.* (2005) suggested that moderate-intensity exercise is as effective as high-intensity exercise in improving insulin sensitivity in sedentary men when 1676 kJ are expended per session. Interestingly, an earlier article published by the same group (the latter) using the same cohort suggested that changes in coronary heart disease risk factors are influenced by exercise intensity after greater improvements in lipid profiles were detected following high-intensity exercise (O'Donovan *et al.*, 2005). A more similar study to the current one observed that the combination of caloric restriction and increased physical activity (independent of intensity) produced similar weight loss and changes in lipids, glucose and insulin metabolism across treatment groups (Nicklas *et al.* 2009). The present study confirms the earlier findings made by Nicklas *et al.* (2009) but also provides novel insight to the area by providing information about the adipose tissue metabolism of inactive and overweight/obese individuals facing a period of negative energy balance induced by caloric restriction and increased physical exercise.

Although the benefits of caloric restriction combined with increased physical activity on human health are well documented, little is known about the impact on the transcriptome of adipose tissue during energy deficit in humans. The adipose organ is a major site for energy storage and one of the main tissue being impacted during a bout of negative energy balance (Capel *et al.*, 2009; Bouchard *et al.*, 2010). Seventeen genes involved in a variety of biological processes such as energy homeostasis (AMPK, LEPTIN, APELIN), glucose metabolism (IRS1&2, PDK4, GLUT 4, ADIPONECTIN), lipid metabolism (ADIPONECTIN, SREBP1c, FAS, PPAR γ , LPL, HSL, VISFATIN) and inflammatory pathways (TNF α , IL18, IL6) were selected part of this investigation. All are involved in various metabolic processes and are expressed in adipose tissue. The caloric restriction and increased physical activity protocol adopted here significantly impacted the expression of some of these genes.

Expression of PDK4 in adipose increased in both groups, highlighting a switch of oxidative fuel from glucose to fatty acids by the adipose tissue. PDK4 is a

mitochondrial protein that is a member of the pyruvate dehydrogenase kinase family, a group of enzymes that inhibit the pyruvate dehydrogenase complex (PDC) by phosphorylating one of its subunits (Rowles *et al.*, 1996). Expression of this gene is regulated by glucocorticoids, retinoic acid and insulin (Kwon & Harris, 2004). During starvation, the activation of PDK4 results in a switch of oxidative fuel use from glucose to fatty acids (Wu *et al.*, 2000; Tsintzas *et al.*, 2006; Jeoung *et al.*, 2006). The decrease in systemic triglycerides and the increase in non-esterified fatty acids reported in the present study further confirm this. A study by Hilderbrandt *et al.* (2003) showed PDK4 to be up-regulated in rat muscle following exercise, highlighting the important function of PDK4 post-exercise and the gradual transition from carbohydrate to non-esterified fatty acid metabolism. Chokkalingam *et al.* (2007) demonstrated that insulin can rapidly suppress PDK4 gene expression in skeletal muscle in healthy humans. The model used in the present study decreased HOMA-IR, HOMA- β and the insulinaemic response to a standard glucose load, whilst increasing ISI similarly in both groups, potentially favouring a switch to fatty acid metabolism. As a result, lipolysis was being up-regulated (\uparrow HSL mRNA) in both groups. Changes in HSL mRNA and protein levels have been shown to be correlated (Jocken *et al.*, 2007) and major determinants of the maximum lipolytic capacity of human adipocytes (Large *et al.*, 1998). HSL plays a central part in lipid metabolism as it catalyses the hydrolysis of triglycerides and diglycerides (Holm *et al.*, 2000). This up-regulation in HSL could also reflect an increase in lipolysis to ensure that adipose is carrying out its primary role (i.e.: to liberate NEFA for metabolism by other tissues). HSL protein expression has been shown to be up-regulated during very-low-calorie diet in obese females (Stich *et al.*, 1997).

SREBP-1c was downregulated to a similar degree in both groups in the present study. SREBP-1c is a transcription factor that regulates the expression of the lipogenic enzyme FAS (Minehira *et al.*, 2003) and our data support this as there was a strong positive correlation between changes in the expression of SREBP-1c and changes in the expression of FAS ($r=0.77$; $P>0.001$). SREBP-1c mediates the effects of insulin and glucose on the regulation of key genes associated with glucose metabolism; there was an inverse correlation between

changes in insulin iAUC and changes in the expression of SREBP-1c ($r=-0.47$; $P=0.03$). Tsintzas *et al.* (2006) showed that SREBP-1c mRNA was down-regulated 2.5 fold in skeletal muscle following forty-eight hours of starvation in healthy males. Conversely, carbohydrate overfeeding increased SREBP-1c mRNA by 25 % and 43 % in the adipose tissue of overweight and lean participants, respectively (Minehira *et al.*, 2004). The combination of caloric reduction and increased physical activity favoured an increase in lipolysis (\downarrow SREBP-1c, \downarrow FAS, \uparrow HSL) independent of exercise intensity.

Several markers of inflammation such as CRP ($\Delta=-0.58 \pm 1.14$ and -0.54 ± 0.44 for the MOD and VIG group, respectively), ALT and whole blood WBC were similarly reduced in both groups following the intervention. The decrease in CRP is consistent with the findings reported by Roberts *et al.* (2006) using a similar protocol in men with diabetes. Campbell *et al.* (2009) achieved a 10 % reduction in CRP following a year-long exercise intervention of moderate-intensity in 115 postmenopausal, overweight or obese, middle-aged, sedentary women. Interestingly, they showed that only participants who decreased body fat by 2 % or more experienced a significant reduction in CRP. Imayama *et al.* (2012) concluded that a reduction in weight ≥ 5 % was necessary to reduce inflammatory biomarkers (CRP, IL-6 & serum amyloid A) in overweight and obese postmenopausal women induced either by caloric restriction, exercise or a combination, irrespectively. This highlights the importance of weight loss or deficit *per se* on these parameters (Church *et al.*, 2010).

We cannot refute the possibility that an intervention over a longer period might have resulted in a different pattern of results between the moderate and vigorous intensity groups. Longitudinal studies are required to confirm that these findings apply to more permanent lifestyle changes. The current model being a combination of caloric restriction and increased physical activity; we are unable to dissociate the benefits derived from each component. Further work is required in order to understand the long-term impact of negative energy balance on adipose tissue and other metabolic health markers.

To conclude, this experiment shows that short-term caloric restriction combined with either vigorous or moderate-intensity physical exercise improves insulin sensitivity, lipid profiles and markers of inflammation. The expression of several key genes in adipose tissue of sedentary and overweight/obese men and postmenopausal women was also positively altered when energy expenditure and caloric deficit were matched. These results confirm the positive effects of combined caloric restriction and increased exercise in sedentary overweight men and women. The benefits of increased physical exercise combined with caloric restriction were independent of exercise intensity

CHAPTER 6

General Discussion

6.1 Overview

Throughout the work in this thesis, I have attempted to use several study designs to address the question of whether physical activity (including structured exercise) is associated with reduced concentrations of blood inflammatory markers and whole-body metabolic improvements and whether this is achieved through its impact on energy balance, body weight/fat mass or physical activity *per se*. Specifically, Chapter 3 addressed the question of whether a lifestyle intervention combining dietary advice with increased physical activity would further improve inflammatory markers compared to dietary advice alone and usual care in patients with newly diagnosed type 2 diabetes. Chapter 4 investigated whether daily vigorous-intensity exercise would counteract the metabolic changes induced by short-term overfeeding and reduced physical activity independent of any net attenuation of energy imbalance in healthy young men. Chapter 5 aimed to determine whether caloric restriction combined with vigorous-intensity exercise would further improve metabolic control and inflammatory markers compared to moderate-intensity exercise in middle-aged, overweight/obese men and postmenopausal women.

A summary of the results of all three experimental chapters is outlined below.

Chapter 3: Diet or diet plus physical activity provide similar positive changes in inflammation in patients with newly-diagnosed type 2 diabetes compared to usual care: The Early ACTID randomised controlled trial

- Motivational unsupervised diet and diet plus physical activity interventions in patients with newly-diagnosed type 2 diabetes lead to reductions in CRP, IL-6 and sICAM-1 with an increase in adiponectin.
- There was no greater benefit from adding physical activity advice to dietary advice.

- Approximately half of the observed effect for the primary outcome (CRP) was explained by the change in weight.
- A relatively modest 2-3 % decrease in weight led to a fall in circulating CRP of approximately 20 %.

Chapter 4: Exercise counteracts short-term overfeeding and reduced physical activity independent of any net attenuation of energy imbalance in healthy young men

- The combination of short-term overfeeding and reduced physical activity induced a state of insulin resistance, hyperinsulinaemia and altered expression of several key genes within adipose tissue.
- The inclusion of a daily vigorous-intensity exercise bout largely prevented these changes from taking place independent of any net effect on energy imbalance.
- Exercise has a profound effect on physiological function even in the face of a considerable energy surplus.

Chapter 5: Caloric restriction combined with moderate or vigorous-intensity exercise provide similar positive changes in metabolic control when energy expenditure and caloric deficit are matched

- Three weeks of caloric restriction combined with either vigorous or moderate-intensity physical exercise improved insulin sensitivity, lipid profiles and markers of inflammation.
- The expression of several key genes in adipose tissue of sedentary and overweight/obese men and postmenopausal women was also positively altered when energy expenditure and caloric deficit were matched.
- These results confirm the positive effects of combined caloric restriction and increased exercise in sedentary overweight men and women.
- The benefits of increased physical exercise combined with caloric restriction were independent of exercise intensity.

6.2 Impact of increased physical activity on inflammatory markers and metabolic control

Epidemiological studies suggest that physical activity is beneficial in reducing markers of inflammation and reducing the risk of developing chronic diseases such as the metabolic syndrome and T2D (Rennie *et al.*, 2003; Fischer *et al.*, 2007; Demakakos *et al.*, 2010). Several studies have investigated whether physical activity combined with dietary advice further improves metabolic and inflammatory markers compared to dietary advice alone. A study in patients with T2D found that diet alone conferred the same benefits as diet combined with exercise (Giannopoulou *et al.*, 2005). Conversely, another study with the same population suggests that diet combined with exercise is more beneficial than diet alone in improving insulin resistance (Yokoyama *et al.*, 2004). Importantly, both studies reported similar weight losses between groups. Findings from studies in overweight/obese participants are equally ambiguous. While Larson-Meyer *et al.* (2006) suggested that diet alone or diet combined with exercise similarly improved insulin resistance, Nicklas *et al.* (2004) showed that diet-induced weight loss resulted in greater reductions in inflammatory markers compared to exercise alone or exercise plus diet. Importantly, the diet group experienced a much greater weight loss. In Chapter 3, our findings suggest that diet combined with exercise confers the same positive changes to inflammatory markers as diet alone. Interestingly, the reduction observed in inflammatory markers in Chapter 3 over a year-long intervention are similar to those observed in Chapter 5 following a 3-week intervention. Both studies resulted in a similar change in body weight, suggesting that weight/fat loss might be the driving factor influencing improvements in inflammatory markers. Findings in Chapter 5 demonstrate that benefits derived from exercise (in combination with caloric restriction) are independent of exercise intensity when energy expenditure and caloric deficit are matched. Exercising at higher intensity for less time is equally beneficial to metabolic control and markers of inflammation than exercising for longer duration at a lower intensity. Whether physical activity positively impacts metabolic control and inflammatory markers through a change in weight or fat mass remains unclear.

6.3 Association between exercise and energy balance

Exercise and physical activity make a major contribution to total energy expenditure and therefore overall energy balance. Western lifestyles are characterised by chronically low levels of physical activity and excessive caloric intake, resulting in positive energy. In order to investigate the benefits from exercise, it is critical to control energy balance in order to tease apart the effects of energy balance from exercise/physical activity. Numerous studies have examined overfeeding of varied duration and composition, with the resultant positive energy balance associated with impaired insulin sensitivity (Wang *et al.*, 2001; Cornier *et al.*, 2006; Brons *et al.*, 2009) and marked alterations in adipose tissue gene expression (Shea *et al.*, 2009; Alligier *et al.*, 2012). In Chapter 4, we demonstrated that a daily bout of vigorous-intensity exercise mostly prevented the negative health effects induced by 7 days of overfeeding and reduced physical activity. Critically, this was independent of any impact on energy (im)balance. Whether this is facilitated by regular glycogen turnover or some other consequence of muscle contraction *per se* remains to be explored. These results demonstrate that exercise has a profound effect on physiological function even in the face of a considerable energy surplus. Although the benefits of exercise in the context of an energy surplus are evident, it remains unclear whether increased physical activity in the context of a caloric deficit confers similar benefits.

6.4 Association between weight loss/gain and key genes within adipose tissue

Given that positive energy balance is a major factor leading to obesity and resultant metabolic disturbances, it is surprising that so few studies have focused on changes in the expression patterns of key genes in adipose tissue during an energy surplus or deficit in healthy individuals considering it is a major site for energy storage. We elected to focus on expression changes within adipose tissue and seventeen genes involved in a variety of biological processes such as energy homeostasis, glucose metabolism, lipid metabolism and inflammatory pathways. In Chapter 4, the impact of the overfeeding and reduced physical activity model had a dramatic impact on the expression of key genes in adipose tissue as shown by the amplitude of the changes reported. For

example, FAS and PDK4 were regulated ~4-fold in the SUR group. In comparison, the same genes were regulated ~2-fold in Chapter 5. Although applied in opposite directions, the actual energy imbalances were similar in both these studies resulting in a weight gain of 2.7 kg in SUR group in Chapter 4 *versus* a weight loss of -2.4 kg on average in Chapter 5. One consideration in the interpretation of these studies is that the study design in Chapter 5 lasted over a three-week period unlike the methods employed in Chapter 4 which was very acute (1 week); this could explain why some of the changes seen at the mRNA level are less pronounced. Also, participants in Chapter 5 had an average fat mass of ~32 kg compared to ~11.5 kg in Chapter 4. This could also potentially explain the reduction in the amplitude of the gene expression changes seen Chapter 5. Interestingly, the same genes that were up-regulated (SREBP1c & FAS) and down-regulated (PDK4 & HSL) in Chapter 4 by a period of positive energy balance were down-regulated (SREBP1c & FAS) and up-regulated (PDK4 & HSL) by a period of negative energy balance in Chapter 5.

6.5 Limitations

Changes in the activity levels of participants within the diet plus activity arm in Chapter 3 were modest (10 min per day compared to 3 min per day for the diet arm). Although it was not the purpose of this study, future interventions with a greater increase in physical activity combined with diet are required to establish whether it would result in greater improvements in inflammatory markers (dose response).

As discussed throughout, due to the general inverse association between body weight and physical activity, it is difficult to tease out the independent role of changes in physical activity or weight on metabolic and inflammatory markers. Findings in Chapter 4 bring new evidence to demonstrate that exercise might be beneficial independent of energy balance. Even though participants in the SUR+EX groups experience a dramatic estimated energy surplus over the course of the week (~66000 kJ), the addition of a daily bout of vigorous-intensity exercise bout prevented/restored most of the metabolic changes seen in the SUR group. This demonstrates that in the short-term at least, exercise can prevent

health outcomes from worsening even in the face of an energy surplus. However, it is possible that these findings would not hold in the long term or in a different population.

The model used in Chapter 5 combined restricted caloric intake with increase physical activity. As it is an extreme short-term intervention, future research should focus on examining whether the improvements in metabolic control and inflammatory markers reported here hold in the long term. The study design used in Chapter 5 does not enable us to conclude whether the same benefits would have been achieved through caloric restriction alone.

Changes in the expression of several genes measured in adipose tissue in Chapter 4 & 5 give us an indication of how metabolism and inflammation are regulated during periods of positive and negative energy balance, respectively. We cannot conclude that the changes reported at the mRNA level would translate to changes at the protein level (apart from AMPK).

6.6 Future work

More studies designed to investigate the benefits of exercise independent of changes in energy balance on inflammatory markers and metabolic control are needed in order to clarify the benefits of physical activity. Findings in Chapter 4 suggest that exercise *per se* can have independent effects, future studies exploring the role played by specific macronutrients is required to confirm this. Analysis of skeletal muscle glycogen content and mRNA would be useful in answering some of the remaining questions. More longitudinal studies are required to confirm whether the findings made in Chapter 4 & 5 apply to a more permanent lifestyle change. Chapter 3 suggests that more work is needed to get patients to become more active if we are to examine the independent effect of exercise on inflammatory markers in people with type 2 diabetes. An interdisciplinary approach targeting behavioural changes should be part of future strategies aimed at improving the health of patients newly diagnosed with type 2 diabetes. Future studies where energy balance is carefully controlled are required in order to clarify the impact of exercise on metabolic control and inflammation.

6.7 Conclusions

Based on the results from this thesis, there is reasonable evidence that exercise *per se* can have independent (i.e., independent of energy balance) effects on metabolic control and inflammation although this is in the context of an extreme short-term intervention. Whether exercise contributes towards the health benefits reported during a bout of negative energy balance is less convincing.

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APPENDIX 1

HEALTH QUESTIONNAIRE

Please answer the following questions in order for us to determine your suitability for our research study

1. Date of birth

2. Are you taking prescribed medication/drugs? If so, please list

3. Are you taking non-prescription medicine/drugs (e.g. aspirin)? If so, please list

4. Have you been diagnosed as having any form of chronic or episodic condition (e.g. Asthma, Arthritis, Type II Diabetes)?

5. Do you have a current illness or have you had an illness in the last 6 weeks (e.g. cold or flu)?

6. Do you or have you ever smoked? Please provide details.

7. How many times do you undergo structured vigorous exercise (i.e., that makes you sweat) for 30 minutes or more per week?

8. Has your weight been stable in the last 6 months?

9. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

10. Has your doctor ever said that you have a heart arrhythmia?

11. Do you know of any reason why you should not do physical activity?

12. Do you suffer from a food intolerance or allergy?

13. Do you take any supplements (multivitamins,...)?

APPENDIX 2

HEALTH QUESTIONNAIRE

Please answer the following questions in order for us to determine your suitability for our research study

1. Date of birth
2. Height and weight
3. Are you taking prescribed medication/drugs? If so, please list
4. Are you taking non-prescription medicine/drugs (e.g. aspirin)? If so, please list
5. Is your doctor currently prescribing drugs (e.g. water pills) for your blood pressure or heart condition?
6. Have you been diagnosed as having any form of chronic or episodic condition (e.g. Asthma, Arthritis, Type II Diabetes)?
7. If you are female, are you post-menopausal (no menstruation for at least one year)?
8. If you are female, are you on hormone replacement therapy?
9. Do you have a current illness or have you had an illness in the last 6 weeks (e.g. cold or flu)?

10. Do you or have you ever smoked? Please provide details.
11. Has your weight been stable in the last 6 months?
12. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
13. Has your doctor ever said that you have a heart arrhythmia?
14. Do you know of any reason why you should not do physical activity?
15. Do you feel pain in your chest when you do physical activity?
16. Do you lose your balance because of dizziness or do you lose consciousness?
17. Do you have a bone or joint problem (back, knee or hip) that could be made worse by a change in your physical activity?
18. Do you undergo structured vigorous exercise (i.e., that makes you sweat) on a regular basis?
19. Do you accumulate more than 30 minutes of exercise, in bouts of 10 minutes or more (including walking, household and garden chores, work related exercise) 5 days a week or more?

20. Have your physical activity patterns changed in the last year (if yes, please describe)?

21. Do you take any supplements (multivitamins,...)?

APPENDIX 3

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT
or GUARDIAN (for participants under the age of majority) _____

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.



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APPENDIX 4

SUR GROUP													
	WEIGHT	WEIGHT	AVERAGE	EI									
	DAY1	DAY8	WEIGHT	AVERAGE	PRO	CHO	FAT	ALC	PRO	CHO	FAT	ALC	
	(kg)	(kg)	(kg)	(kJ/day)	%	%	%	%	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	
PARTICIPANT 13	65.1	67.9	66.5	13513	11.8	56.4	31.7	0.0	1600	7621	4278	0	
PARTICIPANT 9	75.8	78.0	76.9	11740	15.1	49.6	30.0	4.7	1775	5824	3521	551	
PARTICIPANT 8	82.7	87.9	85.3	17284	13.2	61.6	25.1	0.4	2280	10638	4331	60	
PARTICIPANT 7	82.0	84.3	83.2	12557	14.3	45.8	39.8	0.0	1792	5748	4997	0	
PARTICIPANT 2	92.3	94.8	93.6	17791	14.7	53.8	30.2	1.4	2612	9566	5367	256	
PARTICIPANT 4	74.2	75.9	75.1	12511	14.7	49.0	29.5	6.9	1837	6133	3691	863	
PARTICIPANT 3	68.7	71.4	70.1	15976	11.4	48.6	39.8	0.0	1828	7766	6351	0	
PARTICIPANT 25	76.6	79.7	78.2	16810	17.3	53.7	28.9	0.0	2903	9030	4851	0	
PARTICIPANT 18	81.2	84.6	82.9	15800	14.8	50.1	34.0	1.0	2334	7916	5374	160	
PARTICIPANT 2_4	77.5	78.5	78.0	13576	14.0	48.5	34.9	2.8	1898	6577	4732	376	
PARTICIPANT 2_2	73.4	78.2	75.8	14267	17.4	58.7	24.2	0.0	2475	8369	3448	0	
PARTICIPANT 2_6	54.1	56.2	55.2	7073	21.7	61.9	16.5	0.0	1536	4379	1166	0	
PARTICIPANT 2_7	77.4	79.5	78.5	12662	19.5	45.7	33.2	1.8	2470	5784	4201	222	
AVERAGE	75.5	78.2	76.842	13966	15.4	52.6	30.6	1.5	2103	7335	4331	191	
SUR+EX GROUP													
	WEIGHT	WEIGHT	AVERAGE	EI									
	DAY1	DAY8	WEIGHT	AVERAGE	PRO	CHO	FAT	ALC	PRO	CHO	FAT	ALC	
	(kg)	(kg)	(kg)	(kJ/day)	%	%	%	%	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	
PARTICIPANT 16	82.4	82.7	82.6	11430	17.7	44.3	33.7	4.3	2020	5067	3857	488	
PARTICIPANT 15	76.7	79.5	78.1	14020	11.8	51.0	34.4	2.6	1653	7153	4820	358	
PARTICIPANT 1	82.9	84.4	83.7	14585	13.8	52.2	30.6	3.2	2014	7616	4466	471	
PARTICIPANT 19	98.9	100.8	99.9	12926	23.1	49.3	27.6	0.0	2991	6370	3571	0	
PARTICIPANT 23	72.8	73.1	73.0	12566	12.5	56.6	27.2	3.7	1573	7110	3418	462	
PARTICIPANT 21	73.0	74.9	74.0	13475	13.6	51.6	33.3	1.4	1831	6950	4490	183	
PARTICIPANT 6	82.8	86.1	84.5	12478	13.3	44.7	37.2	4.5	1660	5578	4641	562	
PARTICIPANT 22	65.2	65.6	65.4	9536	12.4	47.3	31.1	9.1	1178	4512	2965	872	
PARTICIPANT 24	90.1	91.4	90.8	13722	20.0	42.9	35.6	1.5	2747	5887	4878	207	
PARTICIPANT 2_2	62.6	64.5	63.6	11753	16.9	37.7	39.5	6.0	1985	4430	4642	699	
PARTICIPANT 2_3	86.1	88.8	87.5	15721	18.1	52.4	29.3	0.0	2841	8235	4605	0	
PARTICIPANT 2_8	65.3	66.4	65.9	13358	15.8	44.5	40.0	0.0	2116	5939	5342	0	
AVERAGE	78.2	79.9	79.042	12964	15.7	47.9	33.3	3.0	2051	6237	4308	359	
<p>These calculations are based on the average weight of participants during the intervention. Baseline diet information is derived from the diet analysis (Chapter 2.9). DIT was estimated as 21 % for protein, 15 % for alcohol, 2 % for fat and 8 % of Energy Intake (EI) based on Westerterp (2004). Protein and Alcohol surpluses were estimated by subtracting DIT from EI. These calculations do not take into account protein and alcohol metabolism. Fat surpluses were estimated as EI-DIT-FAT oxidised (RMR)+FAT generated via DNL. Carbohydrate surpluses were estimated as EI-DIT-CHO oxidised (RMR)-CHO converted into FAT-CHO oxidised through daily steps and run. The overall average energy surplus for each group was estimated by calculating the sum of the surpluses associated with each macronutrient (Chapter 4.2.5).</p>													

Appendix 4: Chapter 4 calculated energy surpluses

SUR GROUP													
	% OF	El during		minus			minus				minus	minus	plus
		intervention	PRO	PRO	SURPLUS		DIT	SURPLUS		DIT	RMR OX	DNL	SURPLUS
		(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)
PARTICIPANT 13	1.5	20269	2400	504	1896	0	0	0	6417	128	0	2224	8513
PARTICIPANT 9	1.5	17611	2663	559	2104	826	124	702	5281	106	842	0	4334
PARTICIPANT 8	1.5	25926	3420	718	2701	91	14	77	6497	130	0	2730	9097
PARTICIPANT 7	1.5	18836	2688	564	2123	0	0	0	7495	150	0	824	8169
PARTICIPANT 2	1.5	26686	3918	823	3095	384	58	327	8051	161	0	1372	9262
PARTICIPANT 4	1.5	18767	2755	579	2176	1295	194	1101	5536	111	936	0	4490
PARTICIPANT 3	1.5	23965	2742	576	2166	0	0	0	9526	191	2114	0	7222
PARTICIPANT 25	1.5	25215	4355	914	3440	0	0	0	7277	146	0	820	7952
PARTICIPANT 18	1.5	23701	3501	735	2765	239	36	203	8061	161	0	93	7992
PARTICIPANT 2_4	1.5	20363	2847	598	2249	564	85	479	7099	142	4175	0	2782
PARTICIPANT 2_2	1.5	21400	3713	780	2933	0	0	0	5172	103	823	0	4246
PARTICIPANT 2_6	1.5	10609	2304	484	1820	0	0	0	1748	35	582	0	1131
PARTICIPANT 2_7	1.5	18993	3706	778	2927	332	50	283	6302	126	3510	0	2666
AVERAGE	1.5	20949	3155	662	2492	287	43	244	6497	130	999	620	5989
SUR+EX GROUP													
	% OF	El during		minus			minus				minus	minus	plus
		intervention	PRO	PRO	SURPLUS		DIT	SURPLUS		DIT	RMR OX	DNL	SURPLUS
		(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)
PARTICIPANT 16	1.78	20346	3595	755	2840	869	130	738	6865	137	3378	0	3349
PARTICIPANT 15	1.70	23834	2810	590	2220	608	91	517	8194	164	0	1378	9408
PARTICIPANT 1	1.74	25379	3505	736	2769	820	123	697	7771	155	1246	0	6369
PARTICIPANT 19	1.80	23267	5384	1131	4253	0	0	0	6429	129	2272	0	4028
PARTICIPANT 23	1.75	21990	2753	578	2175	809	121	688	5981	120	4396	0	1466
PARTICIPANT 21	1.73	23312	3168	665	2503	317	48	269	7767	155	747	0	6865
PARTICIPANT 6	1.78	22211	2954	620	2334	999	150	850	8260	165	2081	0	6013
PARTICIPANT 22	1.79	17070	2108	443	1665	1560	234	1326	5307	106	4027	0	1174
PARTICIPANT 24	1.76	24151	4835	1015	3820	365	55	310	8586	172	6321	0	2093
PARTICIPANT 2_2	1.74	20450	3454	725	2729	1217	183	1034	8078	162	2813	0	5104
PARTICIPANT 2_3	1.75	27512	4971	1044	3927	0	0	0	8058	161	4687	0	3209
PARTICIPANT 2_8	1.71	22842	3618	760	2858	0	0	0	9134	183	3822	0	5130
AVERAGE	1.75	22697	3596	755	2841	630	95	536	7536	151	2983	115	4517

Appendix 4: Chapter 4 calculated energy surpluses

SUR GROUP		minus	minus	minus	minus	minus		
		DIT	RMR OX	Lipid conv	Steps	Run	SURPLUS	OVERALL
	CHO	CHO	CHO	CHO	CHO	CHO	CHO	ENERGY
	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)
PARTICIPANT 13	11432	915	5563	1802	542	0	2610	13019
PARTICIPANT 9	8737	699	6795	0	653	0	589	7729
PARTICIPANT 8	15957	1277	7096	2212	536	0	4836	16712
PARTICIPANT 7	8621	690	7617	668	635	0	-988	9305
PARTICIPANT 2	14349	1148	8318	1111	667	0	3105	15788
PARTICIPANT 4	9200	736	6321	0	627	0	1515	9282
PARTICIPANT 3	11649	932	5723	0	493	0	4502	13889
PARTICIPANT 25	13546	1084	8284	0	481	0	3697	15089
PARTICIPANT 18	11874	950	9128	75	622	0	1099	12060
PARTICIPANT 2_4	9866	789	4072	0	290	0	4714	10225
PARTICIPANT 2_2	12553	1004	7766	0	554	0	3229	10408
PARTICIPANT 2_6	6569	526	4824	0	409	0	810	3762
PARTICIPANT 2_7	8676	694	5025	0	817	0	2140	8016
AVERAGE	11002	880	6656	451	564	0	2451	11176
SUR+EX GROUP		minus	minus	minus	minus	minus		
		DIT	RMR OX	Lipid conv	Steps	Run	SURPLUS	OVERALL
	CHO	CHO	CHO	CHO	CHO	CHO	CHO	ENERGY
	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)	(kJ/day)
PARTICIPANT 16	9019	722	4810	0	704	2732	52	6979
PARTICIPANT 15	12160	973	7136	1117	550	2665	-280	11864
PARTICIPANT 1	13253	1060	6188	0	618	3151	2235	12070
PARTICIPANT 19	11466	917	6936	0	596	3281	-264	8018
PARTICIPANT 23	12442	995	2966	0	515	2908	5058	9387
PARTICIPANT 21	12024	962	7221	0	547	2916	378	10015
PARTICIPANT 6	9928	794	5416	0	712	3210	-204	8993
PARTICIPANT 22	8076	646	2273	0	476	2560	2121	6286
PARTICIPANT 24	10361	829	2853	0	691	3327	2661	8883
PARTICIPANT 2_2	7708	617	3419	0	470	2602	600	9467
PARTICIPANT 2_3	14411	1153	4978	0	536	3616	4128	11265
PARTICIPANT 2_8	10155	812	3739	0	374	2652	2578	10566
AVERAGE	10917	873	4828	93	566	2968	1588	9483

APPENDIX 5

SUR GROUP												
	WEIGHT	WEIGHT	WEIGHT	AVERAGE	SURPLUS	SURPLUS	SURPLUS	SURPLUS	WEIGHT	WEIGHT	WEIGHT	WEIGHT
	DAY1	DAY8	DIFF	WEIGHT	FAT	FAT	CHO	CHO	CHANGE	CHANGE	CHANGE	GAIN
	(kg)	(kg)	(kg)	(kg)	(g/day)	(g/week)	(g/day)	(g/week)	1g:2.7g ratio	1g:3.3g ratio	1g:4g ratio	(FAT+CHO)
PARTICIPANT 13	65.1	67.9	2.8	66.5	228	1598	166	1163	4303	5001	5815	2761
PARTICIPANT 9	75.8	78.0	2.2	76.9	116	814	37	262	971	1129	1312	1076
PARTICIPANT 8	82.7	87.9	5.2	85.3	244	1708	308	2155	7972	9265	10773	3862
PARTICIPANT 7	82.0	84.3	2.3	83.2	219	1533	-63	-440	-1628	-1892	-2200	1093
PARTICIPANT 2	92.3	94.8	2.5	93.6	248	1739	198	1383	5118	5947	6916	3122
PARTICIPANT 4	74.2	75.9	1.7	75.1	120	843	96	675	2497	2902	3374	1518
PARTICIPANT 3	68.7	71.4	2.7	70.1	194	1356	287	2006	7421	8624	10028	3361
PARTICIPANT 25	76.6	79.7	3.1	78.2	213	1493	235	1647	6094	7082	8235	3140
PARTICIPANT 18	81.2	84.6	3.4	82.9	214	1500	70	489	1811	2104	2447	1990
PARTICIPANT 2_4	77.5	78.5	1.0	78.0	75	522	300	2100	7771	9031	10501	2622
PARTICIPANT 2_2	73.4	78.2	4.8	75.8	114	797	205	1438	5322	6185	7192	2235
PARTICIPANT 2_6	54.1	56.2	2.1	55.2	30	212	52	361	1335	1552	1805	573
PARTICIPANT 2_7	77.4	79.5	2.1	78.5	71	500	136	953	3528	4100	4767	1454
AVERAGE	75.5	78.2	2.8	76.8	161	1124	156	1092	4040	4695	5459	2216
SUR+EX GROUP												
	WEIGHT	WEIGHT	WEIGHT	AVERAGE	SURPLUS	SURPLUS	SURPLUS	SURPLUS	WEIGHT	WEIGHT	WEIGHT	WEIGHT
	DAY1	DAY8	DIFF	WEIGHT	FAT	FAT	CHO	CHO	CHANGE	CHANGE	CHANGE	GAIN
	(kg)	(kg)	(kg)	(kg)	(g/day)	(g/week)	(g/day)	(g/week)	1g:2.7g ratio	1g:3.3g ratio	1g:4g ratio	(FAT+CHO)
PARTICIPANT 16	82.4	82.7	0.3	82.6	90	799	3	23	85	99	115	822
PARTICIPANT 15	76.7	79.5	2.8	78.1	252	2245	-18	-125	-462	-537	-624	2120
PARTICIPANT 1	82.9	84.4	1.5	83.7	171	1520	142	996	3684	4281	4978	2516
PARTICIPANT 19	98.9	100.8	1.9	99.9	108	961	-17	-118	-435	-506	-588	844
PARTICIPANT 23	72.8	73.1	0.3	73.0	39	350	322	2253	8338	9690	11267	2603
PARTICIPANT 21	73.0	74.9	1.9	74.0	184	1638	24	168	622	723	841	1807
PARTICIPANT 6	82.8	86.1	3.3	84.5	161	1435	-13	-91	-336	-390	-454	1344
PARTICIPANT 22	65.2	65.6	0.4	65.4	31	280	135	945	3496	4063	4724	1225
PARTICIPANT 24	90.1	91.4	1.3	90.8	56	499	169	1186	4387	5098	5928	1685
PARTICIPANT 2_2	62.6	64.5	1.9	63.6	137	1218	38	267	989	1150	1337	1485
PARTICIPANT 2_3	86.1	88.8	2.7	87.5	86	766	263	1839	6804	7907	9194	2605
PARTICIPANT 2_8	65.3	66.4	1.1	65.9	138	1224	164	1148	4249	4938	5742	2373
AVERAGE	78.2	79.9	1.6	79.0	121	1078	101	708	2618	3043	3538	1786
<p>We are assuming that fat isn't stored with water being hydrophobic and a change in the stromal vascular fraction of the adipose tissue is unlikely within a week. Overall, we are probably underestimating the fat surplus as it's likely that less fat has been oxidised throughout the week taking into account that the RER values were collected after an overnight fast and it's also likely that more fat has been synthesised through DNL for the same reason. Overall, we are probably overestimating the CHO surplus as it's likely that more CHO has been oxidised throughout the week seeing as the RER values were collected after an overnight fast and it's also likely that more CHO has been converted into fat through DNL for the same reason. The CHO utilised for the ~4000 steps is probably an underestimation. This spreadsheet presents 3 possible weight gains associated with the CHO surplus calculated. The first one is based on a previously commonly accepted muscle glycogen to water ratio of 1.0 g:2.7 g (Sherman <i>et al.</i>, 1982). The second one is based on a conversion factor of 4.3 on the assumption of 77 % water content (Piehl Aulin <i>et al.</i>, 2000). The third one is based on the upper limit of 3-4 g of water bound with each gram of glycogen suggested by Olsson & Saltin (1970). The final column shows the weight change that could be expected based on the weekly fat and CHO surpluses (without any water associated). These assumptions do not take into account protein and alcohol metabolism.</p>												